Mechanisms underlying subthreshold and suprathreshold responses in dorsal cochlear nucleus cartwheel cells

Mingjie Tong

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A Dissertation

Entitled

Mechanisms underlying subthreshold and suprathreshold responses in dorsal cochlear nucleus cartwheel cells

by

Mingjie Tong

Submitted as partial fulfillment of the requirements for
the Doctor of Philosophy Degree in
Engineering

_____________________
Advisor: Dr. Scott C. Molitor

_____________________
Graduate School

The University of Toledo

December 2005
An Abstract of

Mechanisms underlying subthreshold and suprathreshold responses in dorsal cochlear nucleus cartwheel cells

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Cartwheel cells (CWCs) are a group of interneurons in the dorsal cochlear nucleus (DCN). Unlike other DCN neurons, which respond to stimuli with simple spikes, or trains of individual action potentials, CWCs respond to suprathreshold stimuli with complex spikes, rapid bursts of action potentials superimposed on a slow depolarization, or a combination of simple and complex spikes. In vitro whole-cell current clamp recordings from young rats (P11 – P15) show that CWCs respond to brief suprathreshold stimuli with complex spikes or simple spikes followed by afterdepolarizations (ADPs); and to subthreshold stimuli with subthreshold depolarizations (SDPs). Although complex spikes
and ADPs are produced by Ca\(^{2+}\) currents, SDPs are unaffected by Ca\(^{2+}\) current antagonists but are substantially depressed by the Na\(^+\) channel antagonist TTX. Voltage clamp recordings reveal that SDPs are produced by a persistent Na\(^+\) current activating below spike threshold in CWCs. A hyperpolarization-activated inward current (I\(_h\)) is shown to contribute to the repolarization of SDPs; and voltage-gated K\(^+\) currents, including transient and Ca\(^{2+}\)-activated K\(^+\) currents contribute to the repolarization of suprathreshold responses in CWCs.

Computational simulations demonstrated that SDPs are produced by persistent Na\(^+\) currents; furthermore, these currents are required for the activation of high-threshold Ca\(^{2+}\) channels during suprathreshold responses to produce complex spikes in response to brief suprathreshold stimuli. Delayed-rectifier, transient and Ca\(^{2+}\)-activated K\(^+\) currents contribute to the repolarization of complex spikes and increase of maximal conductances in any of three K\(^+\) currents converts complex spikes into simple spikes. In contrast, removal of the hyperpolarization-activated inward current I\(_h\) increases the amplitude and duration of SDPs, but otherwise has little effect on suprathreshold responses of CWCs. These results demonstrate that a combination of voltage-gated conductances that activate over a range of subthreshold and suprathreshold membrane potentials contribute to the unique electrophysiologic responses of CWCs.
# Table of Contents

Abstract iii

Table of contents v

List of figures viii

List of tables x

1 Introduction 1

1.1 Dorsal Cochlear Nucleus 3

1.2 Complex spiking cartwheel cells (CWCs) in DCN 6

1.3 Complex spiking neurons in the central nervous system 12

1.4 Suprathreshold responses in cartwheel cells 13

1.5 Subthreshold responses in cartwheel cells 15

2 Materials and Experimental Methods 17

2.1 Electrophysiology 17

2.1.1 Preparation of slices 17

2.1.2 Solutions 18

2.1.3 Electrophysiologic recordings 21

2.1.4 Morphologic identification 22

2.2 Data analysis 23

2.2.1 Isolation of SDPs 23

2.2.2 Voltage dependence of SDPs 30
2.2.3  $I_h$ analysis on voltage-clamp recordings  

2.3  Simulation methods  
   2.3.1  Simulation Model  
   2.3.2  Active conductances  
   2.3.3  Clamp protocols  

3  Experimental results  
   3.1  CWCs generate both complex spikes and simple spikes  
   3.2  Subthreshold responses of CWCs  
   3.3  Distinct mechanisms underlie ADPs and SDPs  
   3.4  Hyperpolarization-activated cation current ($I_h$) in CWCs  
   3.4.1  $I_h$ contributes to repolarizing phase of SDPs in CWCs  
   3.4.2  Activation properties of $I_h$ in CWCs  
   3.5  Voltage-gated Na$^+$ currents in CWCs  
   3.5.1  SDPs are mediated by voltage-gated Na$^+$ current  
   3.5.2  Voltage dependence of persistent Na$^+$ current  
   3.6  K$^+$ currents in CWCs  
   3.6.1  Voltage-gated K$^+$ currents in CWCs  
   3.6.2  Ca$^{2+}$-activated K$^+$ currents in CWCs  
   3.7  Pre-step voltage dependence of CWCs  

4  Simulation results  
   4.1  Ionic currents in model of CWCs  
   4.1.1  Fast Na$^+$ current ($I_{Na}$)  
   4.1.2  Persistent Na$^+$ current ($I_{NaP}$)
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Auditory pathways in DCN</td>
<td>4</td>
</tr>
<tr>
<td>1-2</td>
<td>Firing patterns of DCN neurons</td>
<td>8</td>
</tr>
<tr>
<td>1-3</td>
<td>Firing patterns of DCN PCs modulated by inhibitory synaptic inputs</td>
<td>10</td>
</tr>
<tr>
<td>2-1</td>
<td>Preparation of brainstem slices containing DCN</td>
<td>19</td>
</tr>
<tr>
<td>2-2</td>
<td>Fluorescence images of DCN neurons</td>
<td>24</td>
</tr>
<tr>
<td>2-3</td>
<td>Effects of membrane resistance ($R_m$) on the isolation of subthreshold</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>depolarizations (SDPs)</td>
<td></td>
</tr>
<tr>
<td>3-1</td>
<td>Suprathreshold responses of CWCs</td>
<td>37</td>
</tr>
<tr>
<td>3-2</td>
<td>Suprathreshold and subthreshold responses of neurons in the dorsal</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>cochlear nucleus (DCN)</td>
<td></td>
</tr>
<tr>
<td>3-3</td>
<td>Voltage dependence of and $R_m$ changes during SDPs obtained from</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>CWCs</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>Role of Ca$^{2+}$ channels in suprathreshold and subthreshold responses of</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>CWCs</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>ZD7288 augments subthreshold responses</td>
<td>49</td>
</tr>
<tr>
<td>3-6</td>
<td>SDP magnitudes are not altered by changes in the resting $V_m$</td>
<td>52</td>
</tr>
<tr>
<td>3-7</td>
<td>Activation of $I_h$</td>
<td>55</td>
</tr>
<tr>
<td>3-8</td>
<td>SDPs are partially blocked by TTX</td>
<td>58</td>
</tr>
<tr>
<td>3-9</td>
<td>Anticonvulsants phenytoin and riluzole have no effect on SDP magnitudes</td>
<td>61</td>
</tr>
<tr>
<td>3-10</td>
<td>Na$^+$ current elicited by a slow ramp protocol in CWCs</td>
<td>63</td>
</tr>
</tbody>
</table>
3-11 4-AP (1 mM) produces complex spiking responses in simple spiking CWCs, but not in pyramidal cells 66

3-12 Voltage-gated Ca$^{2+}$ current antagonists Ni$^{2+}$/Cd$^{2+}$ widened the half-width of APs 69

3-13 Pre-step voltage dependence of CWCs 72

4-1 Kinetic properties of Fast Na$^+$ current 77

4-2 Kinetic properties of persistent Na$^+$ current 80

4-3 Kinetic properties of N-type voltage-gated Ca$^{2+}$ current 83

4-4 Kinetic properties of I_h 86

4-5 Kinetic properties of delayed-rectifier K$^+$ current 89

4-6 Kinetic properties of transient K$^+$ current 92

4-7 Kinetic properties of Ca$^{2+}$-activated K$^+$ current 95

4-8 Comparison between experimental and modeling results 98

4-9 Persistent Na$^+$ current (I_{NaP}) in CWCs 101

4-10 Persistent Na$^+$ current affects suprathreshold responses in CWCs by changing V_{half} of steady-state activation curve 104

4-11 N-type voltage-gated Ca$^{2+}$ current modulates suprathreshold responses in CWC model 108

4-12 Transient K$^+$ current contributes to the repolarization of suprathreshold responses in CWCs 111

4-13 I_h contributes to repolarization of SDPs 113

5-1 I_h affects both subthreshold and suprathreshold responses in CWCs when V was depolarized 121
List of Tables

1 Comparison of Na+ mediated APs obtained from CWCs in adult (> 1 month old) guinea pig and in P11 – P16 rat DCN.
Chapter One

Introduction

Cartwheel cells (CWCs) are a group of interneurons in the dorsal cochlear nucleus (DCN), part of the brainstem complex that receives inputs from auditory nerve fibers ascending from the cochlea. Unlike other DCN neurons, which respond to excitatory stimuli with individual or repetitive trains of action potentials (APs) referred to as simple spikes, CWCs respond with complex spikes, or rapid bursts of action potentials superimposed on a slow depolarization (Manis et al. 1994; Zhang and Oertel 1993). Though characterized as complex spiking neurons, CWCs also respond spontaneously (Waller and Godfrey 1994) to acoustic stimulation (Davis and Young 1997; Ding et al. 1999; Parham and Kim 1995a) and to intracellular current injection (Ding and Voigt 1997) with a combination of simple and complex spikes. Because CWCs provide inhibition to pyramidal cells (PCs) that form the major projection of the DCN to the inferior colliculus, the ability of CWCs to respond to excitation with simple or complex spikes could have a profound impact upon the information transmitted by the DCN to higher auditory centers.

Although it is known that Ca$^{2+}$ channels mediate the slow depolarization that underlies complex spiking (Agar et al. 1996; Golding and Oertel 1997), little is known
about the underlying mechanisms that determine whether CWCs will respond with simple spikes or complex spikes. Given the absence of low-threshold Ca$^{2+}$ currents from DCN neurons (Molitor and Manis 1999), it is not clear how CWCs respond with complex spikes to low-threshold stimuli. To understand the electrophysiologic mechanisms that underlie complex-spiking, the research described with this thesis utilize whole-cell recordings to examine the responses of CWCs to depolarizing stimuli at and below spike threshold. This research also demonstrated that the CWCs respond to subthreshold stimuli with a slow active event, or a subthreshold depolarization (SDP), that appears as a characteristic deviation from the exponential decay of the membrane potential following the offset of a brief current pulse. Subsequent studies revealed that SDPs were produced by non-inactivating (or persistent) Na$^{+}$ currents activated at subthreshold membrane potentials. Modeling results suggested that the sustained activation of these subthreshold Na$^{+}$ currents is required to activate higher-threshold Ca$^{2+}$ currents that underlie complex spikes. Additional experiments and modeling results showed that a hyperpolarization-activated inward current ($I_{h}$) repolarizes the subthreshold responses of CWCs, and 4-aminopyridine sensitive K$^{+}$ currents can change the suprathreshold response from complex spiking to simple spiking. Therefore, the ability of CWCs to respond with complex spikes results from the interaction of subthreshold Na$^{+}$ and suprathreshold Ca$^{2+}$ currents; $I_{h}$ and various 4-AP sensitive K$^{+}$ currents may then play a role in regulating the ability of CWCs to produce simple or complex spikes.
1.1. Dorsal Cochlear Nucleus

The cochlear nucleus (CN) in the brainstem represents the first level of auditory processing in the central nervous system. Based on anatomical distinctions, the CN can be divided into two subnuclei, DCN and ventral cochlear nucleus (VCN), both of which receive inputs from auditory nerve fibers (ANFs) and project to higher auditory centers. Whereas the VCN seems to be part of the core auditory pathway, extracting various information about sound properties, the DCN may have much more complex processing functions, as it integrates auditory and non-auditory information through a cerebellar-like circuit and displays non-monotonic functions to acoustic stimuli (Nelken and Young 1996; Oertel and Young 2004). There are two anatomically and functionally distinct afferent pathways in DCN (Fig. 1-1). One pathway resides within the deep layers of the DCN, carrying auditory information through ANFs to the basal dendrites of PCs, the major projection of the DCN to the inferior colliculus. The other pathway resides within the superficial molecular layer, carrying multisensory information to the apical dendrites of PCs through parallel fibers (PFs), the axons of granule cells (GCs) found throughout the cochlear nucleus. GCs are innervated by mossy fibers (MFs) originating from various auditory and non-auditory sources, including somatosensory (Itoh et al. 1987; Weinberg and Rustioni 1987; Wright and Ryugo 1996); vestibular (Burian and Gstoettner 1988; Kevetter and Perachio 1989); and descending auditory pathways (Brown et al. 1988; Caicedo and Herbert 1993; Weedman and Ryugo 1996). Various interneurons also contribute to the processing in this circuit, including CWCs, which receive PF input and provide inhibition to PCs.
**Figure 1-1:** *Auditory pathways in DCN.* In deeper layers (Layer III), auditory nerve fibers (ANFs) project to the basal dendrites of pyramidal cells (PCs), a population of projection neurons in the DCN providing outputs to inferior colliculus (IC). Vertical cells (Ve) in the DCN and T and type D stellate cells (T St and D St) of the posteroverentral cochlear nucleus (PVCN) also contribute to the circuitry within deeper layers of the DCN. In the superficial molecular layer (Layer I), parallel fibers (PFs), the axons of granule cells (GCs) contacted by mossy fibers (MFs), project to the apical dendrites of PCs, dendrites of Golgi cells (Go), superficial stellate cells (St) and dendrites of cartwheel cells (CWCs). Modified from Osen and Mugnaini (1981) by Nelken and Young (1996).
1.2. Complex spiking cartwheel cells (CWCs) in DCN

Unlike other neurons of CN, which respond to excitatory inputs with single or repetitive action potentials known as simple spikes (Fig. 1-2 A), CWCs generate complex spikes, which are groups of rapid bursts of action potentials superimposed on a slow depolarization (Fig. 1-2 B). However, in many preparations, CWCs can respond with group of simple spikes or the combination of simple spikes and complex spikes (Agar et al. 1996, 1997; Chen et al. 1999; Davis and Young 1997; Golding and Oertel 1997; Manis et al. 1994; Parham and Kim 1995a; Waller and Godfrey 1994; Waller et al. 1996a; Zhang and Oertel 1993). The ability of CWCs to respond with simple or complex spikes could have profound impact on PC responses and information processing in the DCN.

In addition to regular trains of simple spikes, in vivo recordings from PCs responding to tonal stimuli exhibit buildup responses characterized by a prolonged first spike latency, and pauser responses characterized by a long first interspike interval (Evans and Nelson 1973; Godfrey et al. 1975; Pfeiffer 1966; Rhode and Smith 1986; Young and Brownell 1976). Intracellular recordings from guinea pig DCN in vitro have demonstrated that regular responses are evoked from resting membrane potentials, whereas buildup and pauser responses are evoked from hyperpolarized membrane potentials (Manis 1990). The hyperpolarization serves to remove inactivation from a rapid-inactivating K⁺ channels, which in turn can delay the first spike or prolong the first interspike interval (Kanold and Manis 1999, 2001).

One source of hyperpolarization in PCs may be the inhibitory postsynaptic potentials (IPSPs) produced by the inhibition provided by CWCs (Golding and Oertel
A single IPSP produced by a simple-spiking CWC may not provide sufficient hyperpolarization to convert the suprathreshold responses of PCs from regular to buildup or pauser. A computational model of DCN PCs modified from Kanold and Manis (2001) demonstrated that individual IPSPs produced by simple spikes and IPSP bursts produced by complex spikes had different effects upon the firing patterns of PCs (Fig. 1-3 A). In particular, the first spike latency during the buildup response following an IPSP burst is much longer than the first spike latency following a single IPSP (Fig. 1-3 A). In addition, the effect of the IPSP burst can last long after the IPSPs have decayed back to the resting membrane potential (Fig. 1-3 B), suggesting that both the magnitude and duration of the inhibition of PCs by CWCs is largely determined by the suprathreshold responses of CWCs.
**Figure 1-2:** Firing patterns of DCN neurons.  

**A:** Pyramidal cells respond to depolarizing current injection with simple spikes that form a repetitive train of individual action potentials (APs).  

**B:** Cartwheel cells respond with complex spikes, or bursts of rapid APs superimposed on slow depolarizations. Also notice the appearance of a simple spike between complex spikes.
**Figure 1-3:** Firing patterns of DCN PCs modulated by inhibitory synaptic inputs.

**A:** A somatic model of DCN PC responded to depolarizing current with trains of simple action potentials (APs). A large increase in first spike latencies (FSLs) is observed if a burst of inhibitory synaptic potentials (IPSPs) were provided before the depolarizing current pulse. The effect by a single IPSP on FSL was much smaller. (Gray: bursting IPSP, black: single IPSP. $\Delta t$: time interval between postsynaptic potential and injected stimuli).

**B:** The effect of IPSPs upon subsequent depolarization was observed long after the IPSPs decayed to the resting membrane potential. Plot of FSL vs. $\Delta t$ showed that increases in FSL produced by IPSP burst lasted more than 100 msec following the last IPSP in the burst. Model is modified from Kanold and Manis (2001).
A

25 mV

20 ms

\[ \Delta t \]

B

- ○ bursting IPSP
- × single IPSP
- — no IPSP

first spike latency

\[ \Delta t \]

30 50 70 90 110 130
1.3. Complex spiking neurons in the central nervous system

There are several other types of complex-spiking neurons within the central nervous system, possessing various biophysical mechanisms and participating in wide range of neuronal signaling pathways.

One group of complex-spiking neurons was discovered within the thalamus. The thalamus collects and processes sensory information from other parts of the central nervous system, then conveys it to the cerebral cortex. Thalamocortical relay neurons receive excitation from ascending sensory and descending cortical inputs, receive inhibition from a large number of local interneurons, and project their output to the cortex. In vitro recordings showed that if the neurons were depolarized from the resting potential, a train of fast and simple spikes would be generated. Otherwise, if the depolarizing stimulus was delivered when the neuron was hyperpolarized below the normal resting potential, complex spikes would be observed (Jahnsen and Llinas 1984a). Low-threshold Ca\textsuperscript{2+} channels are responsible for the slow depolarization that underlies complex spike generation; these channels inactivate at resting potentials and require inhibitory input for removal of this inactivation prior to the arrival of excitatory input (Jahnsen and Llinas 1984b; McCormick and Huguenard 1992a).

Another group of complex-spiking neurons are cerebellar Purkinje neurons (Llinas and Sugimori 1980a, b). Purkinje neurons receive excitatory stimuli from two distinct pathways. One is from parallel fibers, which are axon branches of granule neurons receiving information from various sensory and motor sources. The other comes from climbing fibers (CFs) arising from inferior olivary nuclei in the spinal cord. In adult animals, each Purkinje cell receives synaptic input from 150,000-175,000 PFs but only a
single CF (Crepel and Mariani 1976; Kurihara et al. 1997; Lohof et al. 1996; Strata and Rossi 1998). Synaptic inputs from PFs could elicit simple spikes, whereas those from CFs could generate complex spikes. *In vitro* dendritic recordings (Llinas and Sugimori 1980a; Miyakawa et al. 1992) and modeling results (De Schutter and Bower 1994a, b) revealed that activation of dendritic Ca$^{2+}$ spikes by strong and synchronous stimuli from the climbing fiber input generate complex spikes, whereas asynchronous excitatory inputs from parallel fibers, combined with asynchronous inhibitory inputs from basket cells and stellate cells, generate stochastic simple spikes in the soma.

Complex spiking neurons are found in other regions of the brain as well, such as neocortical pyramidal neurons (Schwindt and Crill 1999), subicular pyramidal neurons (Menendez de la Prida et al. 2003; Staff et al. 2000) and hippocampal pyramidal neurons (Masukawa et al. 1982). In neocortical and hippocampal neurons, complex spikes are produced by the activation of dendritic Ca$^{2+}$ during the retrograde propagation of somatic APs and coincident excitatory synaptic input (Jaffe et al. 1994; Larkum et al. 1999; Magee and Carruth 1999; Schwindt and Crill 1999). Additional studies have also revealed that persistent Na$^{+}$ channels (Azouz et al. 1996; Brown and Griffith 1983b; Brumberg et al. 2000; Su et al. 2001) contribute to the sustained depolarization following the initial fast AP mediated by rapid inactivating Na$^{+}$ channels. In subicular pyramidal neurons, a slowly deactivating high-threshold Ca$^{2+}$ current produces a large Ca$^{2+}$ influx during the repolarizing phase of APs to produce a sustained depolarization that results in multiple APs in a complex spike (Jung et al. 2001).

1.4. Suprathreshold responses in cartwheel cells
As with other complex spiking neurons, complex spikes in CWCs are produced by Ca\(^{2+}\) channels (Golding and Oertel 1997); however, none of the existing bursting models applies to CWCs. In contrast to thalamic relay neurons, in which hyperpolarization is required to deinactivate low-threshold Ca\(^{2+}\) channels to generate complex spikes, bursts in CWCs can be elicited at various membrane potentials without any intervening hyperpolarization (Manis et al. 1994). While other bursting neurons require activations of dendritic Ca\(^{2+}\) channels, CWCs only require small depolarization just above spike threshold levels to generate bursts (Manis et al. 1994; Zhang and Oertel 1993), suggesting that a strong activation of dendritic conductances is not required to initiate the complex spike. Unlike Ca\(^{2+}\) channels in subicular pyramidal neurons, Ca\(^{2+}\) channels in isolated DCN neurons have short deactivation time constants that would result in the rapid deactivation of these Ca\(^{2+}\) channels during the repolarizing phase of the initial fast AP, which would prevent a sustained Ca\(^{2+}\) tail current (Molitor and Manis 1999). The rapid deactivation of Ca\(^{2+}\) currents thus can not deliver the sustained slow depolarization required to elicit the subsequent spikes of bursts as in subicular PCs.

Original studies of complex spiking in CWCs did not suggest the presence of persistent Na\(^{+}\) currents in these cells (Manis et al. 1994), but no other evidence exists that would exclude these channels from contributing to the complex electrophysiologic responses of these cells.

Previous studies on CWCs (Molitor, unpublished data) showed that the afterhyperpolarization following complex spikes was abolished by a Ca\(^{2+}\)-free extracellular solution. This is in agreement with studies showing the hyperpolarization following Ca\(^{2+}\) influx during complex spiking is facilitated by a Ca\(^{2+}\)-activated K\(^{+}\)
(K(Ca)) conductance in hippocampal pyramidal neurons (Brown and Griffith 1983a; Lancaster and Adams 1986; Storm 1987) as well as in cerebellar Purkinje cells (Cingolani et al. 2002; De Schutter and Bower 1994b; Gruol et al. 1989; Miyasho et al. 2001; Womack and Khodakhah 2002). A simulation model of CA3 hippocampal neurons demonstrated that increasing the conductance of one type of voltage-dependent K$^{+}$ channel could convert complex spikes into simple spikes (Migliore et al. 1995). In addition to complex spikes, CWCs also respond to brief suprathreshold stimuli with simple spikes that are followed by a characteristic afterdepolarization (ADP) (Manis et al. 1994) that may represent failed complex spikes in which the underlying depolarization does not sufficiently drive the neuron to produce additional APs (Jensen et al. 1996). Therefore, it is reasonable to hypothesize that at least one type of voltage-gated K$^{+}$ current plays a role in regulating the suprathreshold responses in CWCs.

1.5. Subthreshold responses in cartwheel cells

In addition to the suprathreshold firing patterns in CWCs, studies in our lab show that all identified CWCs respond to brief subthreshold stimuli with a slow active event, or a subthreshold depolarization (SDP), which is a characteristic deviation from the exponential decay of the membrane potential following the offset of the current pulse (Tong and Molitor 2002). Study in spinal lamina I neurons showed that intrinsic ionic currents could enhance synaptic integration time by prolonging SDPs (Prescott and De Koninck 2005). The role of SDPs in generating various firing patterns in CWCs has never been investigated, nor have the underlying ionic mechanisms. However, given the fact that complex spikes (or ADPs) could be elicited by just-above-threshold stimuli in
CWCs (Manis et al. 1994; Zhang and Oertel 1993), it is reasonable to speculate that SDPs can modulate suprathreshold responses. There are a number of possible candidates for producing subthreshold responses. One is low-threshold Ca$^{2+}$ current which activates below the threshold of APs. However, complex spikes do not depend on resting membrane potential, and previous studies did not find evidence for low-threshold Ca$^{2+}$ channels in DCN neurons (Molitor and Manis 1999), leading to the hypothesis that the ionic currents producing subthreshold responses in CWCs are different from those producing suprathreshold responses.
Chapter Two

Materials and Experimental Methods

*In vitro* whole-cell recordings were utilized to investigate the subthreshold and suprathreshold response of CWCs. Current clamp recordings were used to examine the properties of SDPs, such as voltage dependence and changes of input resistances, and the possible involvement of ionic currents in generating various firing patterns in CWCs, including Ca\(^{2+}\) currents, Na\(^{+}\) currents, K\(^{+}\) currents and hyperpolarization-activated inward currents. Voltage-clamp recordings were used to investigate the kinetic properties of a persistent Na\(^{+}\) current and a hyperpolarization-activated inward current in CWCs. Based on experimental data, a one-compartment somatic model was then constructed to further examine the relationship between individual ionic currents and various firing patterns in CWCs.

2.1. Electrophysiology

2.1.1. Preparation of slices

Rat pups (age P11 – P16) were anesthetized with ketamine (44 mg/kg) and decapitated, and the brainstem was quickly removed and placed into an artificial
cerebrospinal fluid (ACSF) with reduced Ca\(^{2+}\); kynurenic acid (1 mM) was added to the dissection solution for tissue obtained from pups younger than P13 (see solution compositions below). The cochlear nuclei were cut into 250 µm thick slices in the trans-strial plane using a vibratory microtome (Vibratome, St. Louis MO), perpendicular to the parallel fibers in the superficial DCN (Fig. 2-1), and were placed into an incubation chamber containing the ACSF maintained at 34 °C. Slices remained in incubation at least 1 hour before being transferred to the recording chamber, and were superfused with the ACSF (2 – 4 ml/min) maintained at 32 °C throughout all recordings.

2.1.2. Solutions

The ACSF for incubation and recording contained (in mM) 130 NaCl, 3 KCl, 1.25 KH\(_2\)PO\(_4\), 20 NaHCO\(_3\), 10 glucose, 2.5 CaCl\(_2\) and 1.3 MgSO\(_4\), or 0.2 CaCl\(_2\) and 4 MgSO\(_4\) for the low Ca\(^{2+}\) ACSF used during dissection. Equimolar substitutions of choline Cl and choline HCO\(_3\) for NaCl and NaHCO\(_3\) were utilized to prepare the Na\(^+\) free extracellular solution. Two variations of the ACSF were utilized during voltage clamp experiments to investigate persistent Na\(^+\) currents and hyperpolarization-activated inward currents (I\(_h\)). During persistent Na\(^+\) current experiments, KCl and KH\(_2\)PO\(_4\) were replaced by 4-aminopyridine (4-AP, 1 mM) and TEACl (10 mM). During I\(_h\) experiments, the modified ACSF contained (in mM) 90 NaCl, 4.25 KCl, 35 TEACl, 5 4-AP, 10 glucose, 20 NaHCO\(_3\), 2.5 CaCl\(_2\), 1.3 MgSO\(_4\) and 2 BaCl\(_2\). The ACSF for dissection, incubation and recording was continuously perfused with 95% O\(_2\) - 5% CO\(_2\) atmosphere to maintain a pH near 7.4. The pipette solution contained (in mM) 120 K gluconate, 20 KCl, 2 Na\(_2\) phosphocreatine, 4 MgATP, 10 HEPES, 0.3 NaGTP, 1.1 EGTA, 25 µM Alexa Fluor 488
**Figure 2-1:** Preparation of brainstem slices containing DCN. **A-B:** orientation of the DCN on the rat brainstem, as viewed from the caudal (A) and lateral (B) surface after removal of the overlying cerebellum. Thick dashed line: the strial axis, or the long axis of the DCN, runs from ventrolateral to dorsomedial, with the dorsomedial end being slightly caudal to the ventrolateral end. Numbered sections transverse to the strial axis, or transstrial planes, are shown in more detail in C. D: dorsal. R: rostral. L: lateral. C: caudal. **C:** organization of ANFs and PFs with respect to transstrial sections of the DCN. Magnified views of the numbered transstrial planes shown in A and B. Terminals of individual ANFs arborize within transstrial planes. PFs run perpendicular to transstrial planes, along the strial axis of the nucleus. Modified from (Blackstad et al. 1984).
hydrazide (Na$^+$ salt), pH 7.20 with 8 – 12 mM KOH. For persistent Na$^+$ current experiments, equimolar K gluconate and KCl were substituted by CsMetSO$_3$ and TEACl.

After an initial characterization of cells, the ACSF was supplemented with strychnine (1 - 5 µM), picrotoxin (5 - 10 µM) and kynurenic acid (1 mM) to block ionotropic excitatory and inhibitory synaptic transmission. The voltage-gated channel antagonists CdCl$_2$ (100 µM), NiCl$_2$ (500 µM), TTX (100 nM – 1 µM), phenytoin (100 µM), riluzole (10 µM), and ZD7288 (20 µM) were diluted from concentrated stock solutions prepared in deionized H$_2$O. The stock solutions of picrotoxin and phenytoin also contained NaOH; however, the final concentration of NaOH was less than 100 µM upon dilution. All chemicals were obtained from Sigma-Aldrich (St. Louis MO), except for Alexa Fluor 488 hydrazide (Molecular Probes, Eugene OR), kynurenic acid (Tocris Cookson, St. Louis MO), TTX (Molecular Probes) and ZD7288 (Tocris Cookson).

2.1.3. Electrophysiologic recordings

Individual cells near the slice surface were visualized with infrared video microscopy (MacVicar 1984) on an Olympus BX51W1 fixed-stage upright microscope with a 40X, 0.8 N.A. water immersion objective. Whole-cell recordings were obtained using an Axopatch 200B or a Multiclamp 700A amplifier (Axon Instruments, Union City CA) in fast current clamp mode, while nucleated-patch recordings on persistent Na$^+$ channels were obtained by Axoptach 200B only. Recording traces were filtered at 10 kHz and digitized at 50 kHz with a 12-bit A/D converter (National Instruments, Austin TX) using customized software developed under MATLAB (Mathworks, Natick MA). Recording pipettes were pulled from borosilicate glass capillary tubing (KG33, Garner...
Glass, Claremont CA) and have a final resistance of 5 - 8 MΩ. Recordings were deemed acceptable if $V_m < -50$ mV (corrected for -10 mV junction potential) and access resistance < 25 MΩ immediately after the whole-cell configuration was obtained. If needed, small amounts of hyperpolarizing holding current (typically less than 200 pA) were applied to prevent spontaneous APs. Bridge balance was performed on-line, automatically for data obtained using the Multiclamp 700A, or manually to minimize the voltage change across the capacitive transient for data obtained using the Axopatch 200B. Capacitive transients were blanked off-line; additional filtering was provided off-line using a 1 kHz low-pass digital filter. For voltage clamp experiments, series resistance errors were compensated on-line; compensation up to 50% was typically achieved. Measured junction potentials between the pipette solution and the ACSF (10 mV for K gluconate pipette solution and 7 mV for CsMetSO_3 pipette solution) were subtracted from all voltage traces prior to analysis.

2.1.4. Morphologic identification

Following the termination of electrophysiologic recordings, the recording pipette was withdrawn and fluorescence images were acquired from different regions and focal depths for morphologic reconstruction. A shuttered mercury arc lamp (HBO 100 W, Olympus) was used with a fluorescein filter set having a 465 - 495 nm excitation filter, a 505 nm dichroic, and a 515 – 555 nm emission filter (Chroma Technology, Rockingham VT) to obtain dye fluorescence. The resulting images were collected with a Quantix 57 digital CCD camera (Roper Scientific, Tucson AZ) using customized software developed under MATLAB. A series of images from different focal depths were collected and
flattened into a single 2-D image using an algorithm that finds the most intense pixel along a column formed by all focal planes at any given X, Y coordinate. Montages of these flattened images were then assembled using Photoshop 7.0 (Adobe Systems, San Jose CA) to simultaneously display all visible anatomic features from different regions.

CWCs were identified as medium-sized (long axis of soma 15 - 20 µm) multipolar neurons with ovoid cell bodies and spiny dendrites typically limited to the superficial DCN. The spines characteristic of CWC dendrites were not always visible due to light scatter through the tissue sections; therefore, dendritic branching patterns were examined to further distinguish CWCs from other DCN neurons. CWC dendrites typically branch at wider angles, resulting in distal processes that sometimes curve around or toward the soma (Wouterlood and Mugnaini 1984), whereas the dendrites of other neurons in the superficial DCN, such as PCs and stellate cells, branch at smaller angles, resulting in dendritic processes that radiate outward from the soma (Fig. 2-2). A total of 102 out of 129 CWCs used in this study were positively identified as CWCs from morphologic reconstruction; no fluorescence images were obtained from the remaining 27 cells because of dye leakage following pipette removal. These cells were identified as CWCs based upon similarities in electrophysiologic responses, and from cell appearance under infrared video microscopy prior to recording.

2.2. Data analysis

2.2.1. Isolation of SDPs

Recordings of the membrane potential ($V_m$) time course were obtained during the presentation of brief (2 msec) depolarizing and hyperpolarizing current pulses whose
Figure 2-2: Fluorescence images of DCN neurons. Grayscale photomontages show the morphology of 2 CWCs and 1 PC. Scale bars: 25 um. CWCs have spiny dendrites that project into the molecular layer and curve back toward the cell body. PCs have two sets of dendrites that radiate away from the cell body; these include spiny apical dendrites that project into the molecular layer and smooth basal dendrites that project to deeper layers. ‘E’ on each photo represents the epidemic surface.
amplitudes were varied in 20 – 50 pA steps to elicit a range of subthreshold and suprathreshold $V_m$ responses. The $V_m$ time courses evoked by depolarizing and hyperpolarizing current pulses of the same magnitude were then added to isolate active events by eliminating the contribution of passive membrane properties that produce the characteristic exponential charging and discharging $V_m$ time course evoked by current pulses. In all figures, the results of this trace addition are shown immediately below the original $V_m$ responses to depolarizing and hyperpolarizing current pulses.

The method used to isolate SDPs assumes that the membrane time constants governing the exponential time course of $V_m$ discharge following the offset of injected current pulses remain identical for depolarizing and hyperpolarizing stimuli. Because these time constants are determined in part by the membrane resistance ($R_m$), changes in $R_m$ that occur during the activation or deactivation of voltage-gated conductances will alter the time course of exponential discharge. Differences in the time course of exponential discharge can produce erroneous SDP magnitudes with the addition of $V_m$ traces elicited by depolarizing and hyperpolarizing stimuli.

Potential sources of error in the SDP isolation procedure are illustrated in figure 2-3. In the absence of voltage-gated conductance changes, $R_m$ remains constant and addition of the $V_m$ time course in response to depolarizing and hyperpolarizing stimuli of the same amplitude will cancel to zero (Fig. 2-3, left column). The presence of voltage-gated conductances that activate in response to suprathreshold depolarization, such as the $Na^+$, $K^+$ and $Ca^{2+}$ conductances that generate APs and ADPs, will reduce $R_m$ and the discharge time constant, producing an erroneous hyperpolarization when the depolarizing and hyperpolarizing responses are added (Fig. 2-3, middle column). This will reduce the
**Figure 2-3:** Effects of membrane resistance ($R_m$) on the isolation of subthreshold depolarizations (SDPs). The voltage across a unit resistance in parallel with a unit capacitance was simulated in response to depolarizing and hyperpolarizing current pulses of equal magnitude. Left column: Addition of depolarizing and hyperpolarizing responses is zero when $R_m$ is the same under both conditions. Middle column: reducing the value of $R_m$ by 50% at the offset of the depolarizing pulse increases the rate of $V_m$ decay (compare solid and dotted lines in top row of traces) and results in a small subthreshold hyperpolarization following addition of the depolarizing and hyperpolarizing responses. Right column: reducing the value of $R_m$ by 50% at the offset of the hyperpolarizing pulse results in a small SDP following addition of the depolarizing and hyperpolarizing responses.
\[ \text{depol } R_m = \text{hyperpol } R_m \]

\[ \text{depol } R_m = \text{hyperpol } R_m / 2 \]

\[ \text{hyperpol } R_m = \text{depol } R_m / 2 \]
measured voltage deflection of APs and ADPs following the addition of responses to depolarizing and hyperpolarizing stimuli. However, this does not affect the interpretation of any results from the present study, in which AP and ADP magnitudes were reduced following the application of voltage-gated channel antagonists that would prevent a decrease of $R_m$ in response to a depolarizing stimulus.

The presence of $I_h$ could also affect the SDP magnitudes obtained from the addition of depolarizing and hyperpolarizing $V_m$ traces. $I_h$ will activate in response to increasing hyperpolarization, reducing $R_m$ and the discharging time constant following the offset of a hyperpolarizing stimulus. A decrease in the time constant of the hyperpolarizing discharge will produce an erroneous depolarization when the depolarizing and hyperpolarizing responses are added (Fig. 2-3, right column). Thus, $I_h$ could erroneously augment SDP magnitudes because of the decreased $R_m$ and discharge time constant following the offset of the hyperpolarizing stimulus, and $I_h$ antagonists would therefore decrease SDP magnitudes by preventing this decrease in $R_m$ and discharge time constant. Instead, application of the $I_h$ antagonist ZD7288 had the opposite effect and augmented SDP magnitudes (Fig. 3-5 B-C). This apparent contradiction can be attributed to the slow time course of $I_h$ activation, which was inferred from the effects of ZD7288 on the time course of $R_m$ changes during the SDP (Fig. 3-5 D). These results show that ZD7288 produces little or no difference in $R_m$ values within the first 12 msec of the stimulus onset, where the majority of the decay during $V_m$ discharge would occur following the stimulus offset. Thus, the level of $I_h$ activation would not significantly change over the duration of the $V_m$ transients evoked by brief current pulses, and is not likely to produce the situation illustrated in the last
column of figure 2-3. However, during the experiments when both voltage-gated Ca$^{2+}$ channel antagonists, Ni$^{2+}$ and Cd$^{2+}$, and voltage-gated Na$^{+}$ channel antagonist, TTX were applied, the resting potentials of CWCs hyperpolarized dramatically (around -10 mV). The following hyperbolizing current pulses would probably activate more I$_h$ compared with those in control with much depolarized resting potentials. In that case, I$_h$ might contribute to the TTX-resistant SDPs (Fig. 3-8 D). Unfortunately, this hypothesis could not be tested experimentally because the whole-cell voltage-clamp recordings showed great instability of resting potentials during Ni$^{2+}$/Cd$^{2+}$, TTX and ZD7288 (I$_h$ antagonist) application.

2.2.2. Voltage dependence of SDPs

The voltage dependence of SDPs is shown by plotting SDP magnitudes obtained from the addition of V$_m$ responses to depolarizing and hyperpolarizing stimuli against the value of V$_m$ at the SDP peak during the response to a depolarizing stimulus. For example, if the response to a +1 nA pulse from a holding V$_m$ of -60 mV produces a 7 mV deflection, and the response to a -1 nA pulse produces a -2 mV deflection, then the SDP magnitude plotted on the ordinate would be 7 mV + -2 mV = 5 mV, and the value of V$_m$ plotted on the abscissa would be -60 mV + 7 mV = -53 mV. In figures where traces from the same cell are compared across different pharmacologic manipulations, traces were selected so that the V$_m$ values at the SDP peak were the most similar, and in many cases were evoked by different current amplitudes depending on the changes in R$_m$ produced by the pharmacologic manipulation. To normalize voltage dependence across cells, V$_m$ values plotted on the abscissa were offset so that V$_m$ = 0 mV at AP threshold, and the
SDP magnitudes were binned across a standardized voltage base (1 – 2.5 mV bin widths) prior to averaging across experiments (Fig. 3-3 A-B). No AP threshold could be detected in experiments utilizing TTX ACSF; therefore the original AP threshold under control conditions prior to application of TTX ACSF was used to calculate voltage dependence (Fig. 3-8 D).

2.2.3. *I*<sub>h</sub> analysis on voltage-clamp recordings

Recordings of current traces of *I*<sub>h</sub> with voltage clamp were fitted with a function of the form

\[
I(t) = A_0 + A_1 \exp(-t / \tau_1) + A_2 \exp(-t / \tau_2)
\]

using a Marquardt-Levenberg least-squares algorithm that minimized the errors over five parameters \(A_0, A_1, A_2, \tau_1, \tau_2\). To prevent artifacts from whole-cell capacitance, the initial 20 ~ 50 msec of each current trace was ignored by the fitting procedure.

Activation and deactivation tail currents were used to construct the steady-state activation curve of *I*<sub>h</sub> (Fig. 3-7). Cells were generally held at -50 mV, stepped for 1.4 s to a group of test potentials ranging from around -150 mV to -40 mV to activate and deactivate *I*<sub>h</sub>, and then poststepped to -70mV for 0.5 s. Instantaneous tail currents (*I*<sub>tail</sub>(t = 0)) were computed by extrapolating the current traces back to the starting time of post step using the biexponential fit. Steady-state tail currents (*I*<sub>tail</sub>(t = inf)) were set to the values of tail currents when test potential equaled to the level of post step potential, which is at -70 mV. Difference tail currents (*I*<sub>tail_diff</sub>) were then calculated by subtracting steady-sate tail currents from instantaneous ones (*I*<sub>tail_diff</sub> = *I*<sub>tail</sub>(t = 0) - *I*<sub>tail</sub>(t = inf)). The steady-state activation function *n*<sub>inf</sub>(V) was obtained by a normalized equation as follows.
\[ n_{\text{inf}}(V) = \frac{I_{\text{tail, diff}}(V) - I_{\text{tail, diff}}(V_{\text{min}})}{I_{\text{tail, diff}}(V_{\text{max}}) - I_{\text{tail, diff}}(V_{\text{min}})} \]  

(2)

where \( V_{\text{max}} \) and \( V_{\text{min}} \) represent membrane potentials corresponding to maximal and zero activation of \( g_h \). Finally, activation curves were fit by a Boltzmann function (see equation 6) to provide a functional relationship between steady-state activation and membrane potential \( V_m \).

2.3. Simulation methods

2.3.1. Simulation Model

All simulation work was carried out using NEURON 5.7 (Hines and Carnevale 2001) under the Windows 2000 operating system. Based on experimental estimates, a one compartment model of a CWC was created to represent a cylindrical cell body approximately 31 \( \mu \)m in diameter and 15.4 \( \mu \)m in length. The specific membrane capacitance was set to 1 \( \mu \)F/cm\(^2\), and the cytoplasmic resistivity 35.4 \( \Omega \) cm. The leak conductance was set as 0.1 mS/cm\(^2\), resting potential as -68 mV. The numerical integration used NEURON’s backward-Euler method with a time step of 0.025 msec.

2.3.2. Active conductances

The active conductances in the model used the Hodgkin-Huxley formulation to account for the voltage-dependence and time course of activation and inactivation kinetics (Hodgkin and Huxley 1952). In this formulation, each ionic current could be presented as Ohm’s law

\[ I = g(V - V_{\text{rev}}) \]  

(3)
where \( g \) is the conductance of ionic current and \((V-V_{\text{rev}})\) the voltage driving force, the difference between the existing membrane potential \( V \) and reversal potential \( V_{\text{rev}} \), the membrane potential at which there would be no net electrochemical ionic current. The conductance \( g \) is voltage- and time-dependent, making ionic current voltage- and time-dependent and presented by the following equations:

\[
I(V, t) = G_{\text{max}} m(V, t)^p h(V, t)^q (V - V_{\text{rev}})
\]  

(4)

\[
\frac{ds}{dt} = \frac{(s_{\text{inf}} - s)}{\tau_s}
\]

for \( s = m, h \)

(5)

where \( I(V, t) \) is the ionic current as a function of time \( t \); \( G_{\text{max}} \) is the maximal conductance of current; \( m(V, t) \) and \( h(V, t) \) are activation and inactivation, respectively; \( p, q \) are the numbers of activation and inactivation gates. The variable \( s \) represents either of gating variables \( m \) or \( h \). \( \tau_s \) is the first order time constant for \( m \) or \( h \). The steady-state activation or inactivation \( s_{\text{inf}} \) can be represented as a Boltzmann function

\[
s_{\text{inf}} = \frac{1}{1 + e^{(V - V_{\text{half}})/K_s}}
\]

for \( s = m, h \)

(6)

where \( V_{\text{half}} \) is the activation or inactivation mid-point voltage and \( K_s \) the slope factor of the activation/inactivation curves.

### 2.3.3. Clamp protocols

A simulated ideal two-electrode current-clamp was applied to the somatic compartment. During brief pulse current-clamp simulation, membrane potential was held at -63.4 mV by applying zero injected currents for 200 msec to allow state variables to stabilize. It was then stepped to a series of test pulses for 2 msec to elicit subthreshold and suprathreshold responses. For long pulse current-clamp simulation, the model was
held at the same holding potential but the duration of test pulses was extended to 100 msec.
Chapter Three

Experimental Results

CWCs responded to suprathreshold current injection with complex spikes, or a simple spike followed by a characteristic afterdepolarization (ADP) that is mediated by Ca\(^{2+}\) channels. CWCs responded to subthreshold stimuli with a slow active event, or a subthreshold depolarization (SDP), that appears as a characteristic deviation from the exponential decay of the membrane potential following the offset of the current pulse. SDPs were unaffected by the Ca\(^{2+}\) channel antagonists Cd\(^{2+}\) and Ni\(^{2+}\), the anticonvulsants phenytoin and riluzole, but substantially depressed by TTX. Voltage-clamp recordings showed that a component of TTX-sensitive persistent Na\(^{+}\) current existed in CWCs. SDPs were augmented by ZD7288, which blocked a hyperpolarization-activated inward current (I\(_h\)) in these cells that contributed to the repolarization of the membrane. Voltage-clamp recordings confirmed the existence of I\(_h\) in CWCs.

3.1. CWCs generate both complex spikes and simple spikes

Recordings from 102 identified CWCs were obtained to investigate the ionic mechanisms underlying subthreshold and suprathreshold responses. Cells were initially
characterized using 100 msec current injections from a holding potential around -65 mV to -70 mV. There were two different responses to sustained current injection just above spike threshold. There were 23 complex-spiking CWCs responding to just above threshold depolarizing currents with multiple APs superimposed upon a slow underlying depolarization (Fig. 3-1, left column upper panel), as well as 94 simple-spiking CWCs responding with a simple spike (Fig. 3-1, right column upper panel). While given suprathreshold current injections, complex-spiking CWCs responded with one or more complex spikes (Fig. 3-1, left column middle panel) and simple-spiking CWCs responded with a train of simple spikes (Fig. 3-1, right column middle panel). In 65 of 94 simple-spiking CWCs, complex spikes could be evoked from simple-spiking CWCs by increasing the amplitudes of injected currents (Fig. 3-1, inset of lower panel). Both complex-spiking and simple-spiking CWCs showed similar patterns of decreasing first spike latency (FSL) with increasing injection amplitudes. However, complex-spiking CWCs had short first inter-spike intervals (ISIs) that were constant over the whole range of suprathreshold stimuli while that simple-spiking CWCs showed long ISIs at the beginning of suprathreshold stimuli and shortened as amplitudes increased (Fig. 3-1, lower panel).

3.2. Subthreshold responses of CWCs

To isolate voltage-gated responses from passive membrane potential changes due to current injection, the electrophysiologic responses of CWCs were examined using 2 msec current pulses with amplitudes set just above (Fig. 3-2 A) or just below (Fig. 3-2 B) the threshold for AP initiation. CWCs respond to brief suprathreshold stimuli with one of
Figure 3-1: Suprathreshold responses of CWCs. Responses of 2 CWCs to suprathreshold current injection, one with AP bursts (Left column), one with regular AP trains (Right column). Upper panel: responses to just above threshold depolarizing current injection and corresponding hyperpolarizing stimulus. Middle panel: responses to current injection well above spike threshold and corresponding hyperpolarizing stimulus. Lower panel: First spike latency (FSL) and first interspike interval (ISI) plotted vs. amplitude of injected current. Increasing stimulus levels can elicit AP bursts in simple spiking CWCs (inset).
**Figure 3-2:** *Suprathreshold and subthreshold responses of neurons in the dorsal cochlear nucleus (DCN).*  
**A – B:** Membrane potential ($V_m$) time course in response to suprathreshold (B) and subthreshold current injection. Suprathreshold and subthreshold responses from a simple spiking CWC (far left column), a complex spiking CWC (middle left column), a simple and complex spiking CWC (middle left and right columns) and a PC (far right column) are shown. Top row of $V_m$ traces: $V_m$ time courses evoked by depolarizing and hyperpolarizing current pulses of equal magnitude. Bottom row of $V_m$ traces: $V_m$ time courses evoked by depolarizing and hyperpolarizing current pulses were added to isolate active events. Time course of injected current is shown below both rows of $V_m$ traces. Voltage scale for the original (50 mV) and added (30 mV) $V_m$ traces evoked by suprathreshold stimulus in A applies to the corresponding $V_m$ traces evoked by subthreshold stimulus in B.
two response patterns. The majority of CWCs (90 out of 102 cells) respond to depolarizing current injection with a single AP that is followed by a characteristic afterdepolarization, or ADP (Fig. 3-2 A, far left). A few CWCs (5 out of 102 cells) respond to a brief pulse of depolarizing current with multiple APs superimposed upon a slow underlying depolarization, or a complex spike (Fig. 3-2 A, middle left). The remaining CWCs were classified as having an intermediate response in which simple spikes were evoked by depolarizing current injection just above AP threshold, and complex spikes were evoked by increasing the amplitude of injected current (Fig. 3-2 A, middle right). In contrast, pyramidal cells (PCs) always respond to suprathreshold current injection with simple spikes without ADPs (Fig. 3-2 A, far right). In some recordings (5 out of 7 cells), the addition of $V_m$ traces revealed an AHP that may be attributed to the activation of $K^+$ channels following the repolarizing phase of the AP, or due to changes in $R_m$ that occur during $Na^+$ channel activation (see section 2.7.).

CWCs also respond to subthreshold current injection with active electrophysiologic responses known as subthreshold depolarizations, or SDPs. SDPs were observed as a deviation from the exponential decay of the $V_m$ time course following the offset of depolarizing current injection (Fig. 3-2 B, top row of $V_m$ traces), and were isolated following the addition of responses to depolarizing and hyperpolarizing current injection (Fig. 3-2 B, bottom row of $V_m$ traces). SDPs of varying magnitudes were observed in all CWCs, regardless of whether these cells responded to suprathreshold current injection with simple or complex spikes. Furthermore, no significant differences could be observed in the magnitudes of SDPs evoked just below AP threshold in simple spiking ($7.12 \pm 0.45 \text{ mV, } N = 48$) and complex spiking ($7.55 \pm 0.66 \text{ mV, } N = 32$) CWCs.
In contrast, no SDPs were observed in the subthreshold responses of any PCs (Fig. 3-2 B, far right column). In a few recordings (2 out of 7 PCs), a small subthreshold hyperpolarization was observed following the addition of $V_m$ traces (not shown).

The voltage dependence of SDPs was examined by varying the amplitude of depolarizing current pulses. From a resting $V_m$ around -70 mV, SDPs became more prominent as $V_m$ increased toward AP threshold (Fig. 3-3 A). The data are summarized in Figure 3-3 B, which shows the magnitude of SDPs vs. $V_m$ averaged across 26 CWCs. Because of the variation of AP thresholds across these CWCs (-51.2 ± 1.1 mV), which determines the range of $V_m$ over which SDPs can be observed, the $V_m$ is normalized to AP threshold for each cell so that 0 mV is set at the AP threshold. These data show that SDPs $> 2$ mV in amplitude can be evoked up to 10 mV below AP threshold, and the magnitude of SDPs increases exponentially as $V_m$ approaches AP threshold.

There are two possible mechanisms that could produce a SDP: the opening of depolarization-activated channels that produce inward current, such as voltage-gated Na$^+$ or Ca$^{2+}$ channels, or the closing of hyperpolarization-activated channels that produce outward current, such as anomalous rectifiers. To investigate these two possibilities, a protocol previously used to investigate $R_m$ changes during ADPs elicited by hippocampal CA1 PCs (Jensen et al. 1996) was used to monitor $R_m$ changes throughout the time course of the SDP. To measure $R_m$, a 1 msec pulse of hyperpolarizing current was presented at different intervals following the presentation of a 2 msec pulse of depolarizing current to evoke a SDP (Fig. 3-3 C, top row of traces). The deflection of $V_m$ between the onset and the offset of the 1 msec hyperpolarizing pulse was used as a measure of $R_m$, and was normalized to the deflection of $V_m$ produced by the same
**Figure 3-3:** Voltage dependence of and $R_m$ changes during SDPs obtained from CWCs.  

**A:** $V_m$ time courses (top row of $V_m$ traces) evoked by depolarizing and hyperpolarizing current injection with varying pulse amplitudes. The $V_m$ time courses evoked by depolarizing and hyperpolarizing current pulses are added to isolate active events (bottom row of $V_m$ traces).  

**B:** SDP magnitude vs. $V_m$ averaged across 26 CWCs. The value of $V_m$ plotted along the abscissa is obtained from the original $V_m$ trace evoked by depolarizing current injection at the point where the SDP peak is measured.  

**C:** $R_m$ protocol. Brief hyperpolarizing current pulses are presented at various times after the presentation of a depolarizing current pulse to evoke SDPs (top row of $V_m$ traces). The same hyperpolarizing current pulses are then presented without the depolarizing current pulse to obtain a measure of $R_m$ under resting conditions (bottom row of $V_m$ traces).  

**D:** Normalized $R_m$ changes following a depolarizing stimulus to evoke a SDP (filled circles) and under resting conditions in the absence of a depolarizing stimulus (gray shaded region) plotted vs. the time relative to the onset of the depolarizing stimulus and averaged across 34 CWCs. $V_m$ trace from panel A shown at the top of the graph provides the time course of a SDP for direct comparison to $R_m$ changes. The error bars and width of the shaded region indicate S.E.M. of the averaged $R_m$ changes during the SDP and under resting conditions, respectively.
hyperpolarizing pulse presented without a depolarizing stimulus (Fig. 3-3 C, bottom row of traces). The normalized $R_m$ was then plotted vs. the time of the hyperpolarizing stimulus onset relative to the depolarizing stimulus onset, and averaged across 34 CWCs (Fig. 3-3 D). When compared to the time course of a representative SDP (Fig. 3-3 D, trace above graph), $R_m$ decreases below control levels during the depolarizing phase of the SDP, which indicates channels normally closed at the resting $V_m$ are opening, and suggests that SDPs are produced by a depolarization-activated inward current.

Furthermore, $R_m$ increases above control levels during the repolarizing phase, which indicates channels normally open at the resting $V_m$ are closing. These results suggest that the repolarizing phase of the SDP is not produced by the opening of channels that produce outward current, such as the repolarizing phase of an AP produced by voltage-gated $K^+$ channels, which should decrease $R_m$ below control levels throughout the repolarizing phase. Instead, a set of channels that normally produce an inward current at the resting $V_m$ appear to be closing in response to the depolarizing phase of the SDP, thereby producing the hyperpolarizing drive that is responsible for the repolarizing phase.

**3.3. Distinct mechanisms underlie ADPs and SDPs**

The non-specific $Ca^{2+}$ channel antagonists $Cd^{2+}$ (100 µM) and $Ni^{2+}$ (500 µM) were utilized to investigate the role of voltage-gated $Ca^{2+}$ channels. Suprathreshold responses obtained in the presence of $Cd^{2+}$ and $Ni^{2+}$ (Fig. 3-4 A, top two rows of membrane potential traces) confirmed that the ADPs following simple spikes were generated by $Ca^{2+}$ channels (Agar et al. 1996; Golding and Oertel 1997). Despite the pronounced effects of $Cd^{2+}$ and $Ni^{2+}$ on ADPs, these antagonists had little effect on the
Figure 3-4: Role of $Ca^{2+}$ channels in suprathreshold and subthreshold responses of CWCs.  

**A:** Addition of CdCl$_2$ (100 µM) and NiCl$_2$ (500 µM) reversibly blocks the afterdepolarization (ADP) following an action potential (AP) in response to suprathreshold current injection (top two rows of $V_m$ traces), but has no effect on SDPs (bottom two rows of $V_m$ traces). $V_m$ traces during the application of CdCl$_2$ and NiCl$_2$ were evoked by larger current pulses to facilitate comparison of SDP and ADP magnitudes evoked at similar $V_m$ values, as measured during the response to the depolarizing stimulus. Voltage scale for the original (50 mV) and added (30 mV) suprathreshold $V_m$ traces applies to the corresponding subthreshold $V_m$ traces.  

**B:** Time course of SDP (filled circles) and ADP (open circles) magnitudes during the application of CdCl$_2$ and NiCl$_2$ averaged across 12 CWCs; data from 5 of these 12 CWCs were averaged during washout.  

**C:** SDP magnitude vs. $V_m$ averaged across 6 CWCs before (gray shaded region) and during (filled circles) the application of CdCl$_2$ and NiCl$_2$. 


A. control Cd\textsuperscript{2+} + Ni\textsuperscript{2+} wash

B. Cd\textsuperscript{2+} + Ni\textsuperscript{2+}

C. control Cd\textsuperscript{2+} + Ni\textsuperscript{2+}
SDPs recorded from the same cell (Fig. 3-4 A, bottom two rows of traces). These data are summarized in figure 3-4 B, which show that Cd\(^{2+}\) and Ni\(^{2+}\) produce a complete and reversible block of ADPs elicited by stimuli just above the AP threshold, but have little effect on SDPs elicited by stimuli just below AP threshold. This is also confirmed in figure 3-4 C, which shows that Cd\(^{2+}\) and Ni\(^{2+}\) have little effect on the SDP voltage dependence. Therefore, two distinct ionic currents appear to be responsible for generating ADPs and SDPs.

3.4. Hyperpolarization-activated cation current (I\(_h\)) in CWCs

3.4.1. \(I_h\) contributes to repolarizing phase of SDPs in CWCs

Experiments to examine \(R_m\) changes (Fig. 3-3 C–D) suggest that the repolarization of the SDP is produced by the deactivation of an inward current rather than the activation of an outward current. \(I_h\) is a potential candidate for this current; the deactivation of \(I_h\) is responsible for the repolarizing phase of subthreshold oscillations of stellate cells in the entorhinal cortex (Dickson et al. 2000a, b). Application of the \(I_h\) antagonist ZD7288 (20 \(\mu\)M) hyperpolarized the resting \(V_m\) of 6 CWCs (-59.4 ± 0.6 mV in control, -80.6 ± 1.6 mV in ZD7288) and eliminated the sag in \(V_m\) elicited by a sustained pulse of hyperpolarizing current (Fig. 3-5), indicating that \(I_h\) is present in these cells. ZD7288 reversibly augmented the SDP magnitude over a large range of subthreshold \(V_m\) (Fig. 3-5 B-C). Analysis of \(R_m\) throughout the SDP time course (Fig. 3-5 D) shows that ZD7288 prevents \(R_m\) increases above control levels during the repolarizing phase of the SDP, thereby preventing the closure of channels that would otherwise be normally open at the resting \(V_m\). These results suggest that \(I_h\) contributes to the repolarizing phase of the
**Figure 3-5:** **ZD7288 augments subthreshold responses.**  

**A:** $V_m$ time course elicited by 80 ms depolarizing and hyperpolarizing current pulses under control conditions (top row of $V_m$ traces) and in the presence of 20 µM ZD7288 (bottom row of $V_m$ traces).  

**B:** ZD7288 has little effect on responses to brief pulses of suprathreshold current injection (top two rows of $V_m$ traces), but does increase the magnitude of the SDP evoked by subthreshold current injection (bottom two rows of $V_m$ traces).  

**C:** SDP magnitudes vs. $V_m$ averaged across 8 CWCs under control conditions (gray shaded region) and in the presence of ZD7288 (filled circles).  

**D:** Normalized $R_m$ changes following a depolarizing stimulus to evoke a SDP under control conditions (filled circles) and in the presence of ZD7288 (open circles) plotted vs. the time relative to the onset of the depolarizing stimulus and averaged across 6 CWCs. Also shown is $R_m$ under resting conditions in the absence of a depolarizing stimulus (gray shaded region).  

$V_m$ traces from panel B shown at the top of the graph provide the time course of SDPs under control conditions (dashed line) and in the presence of ZD7288 (solid line) for direct comparison to $R_m$ changes.
SDP by deactivating in response to the depolarizing phase of the SDP, thus reducing the amount of inward current during SDP repolarization that would otherwise be available at the resting $V_m$.

Because the block of $I_h$ by ZD7288 also resulted in a hyperpolarized resting $V_m$, it is possible that the effect of ZD7288 on SDP magnitudes is not directly caused by the block of $I_h$, but rather produced by the secondary effect of changes in the resting $V_m$. For example, if the Na$^+$ channels that generate the SDP inactivate, an increase in SDP magnitudes during exposure to ZD7288 could be directly attributed to the hyperpolarized resting $V_m$. To investigate this possibility, SDPs were elicited from a range of $V_m$ by increasing the amount of hyperpolarizing holding current before and after the presentation of the brief depolarizing pulse to evoke SDPs. To evoke SDPs from a similar range of $V_m$, the magnitude of the depolarizing stimulus was increased to offset the hyperpolarized resting $V_m$. However, no significant increases in SDP magnitudes were observed with a hyperpolarized resting $V_m$ in control conditions and during exposure to ZD7288 (Fig. 3-6 A-C). Increases in the SDP half width (Fig. 3-6 D) and peak time (Fig. 3-6 E) also were observed during the application of ZD7288 over the range of resting $V_m$ that was examined, which further supports the hypothesis that $I_h$ contributes to the repolarization of the SDP.

3.4.2. Activation properties of $I_h$ in CWCs

Whole-cell voltage clamp protocols were used to investigate the activation properties of $I_h$ in CWCs. Modified ACSF with 5 mM 4-AP, 35 mM TEACl and 2 mM
**Figure 3-6**: *SDP magnitudes are not altered by changes in the resting $V_m$. A – B:* Responses to subthreshold stimuli evoked from a range of resting $V_m$ under control conditions (A) and in the presence of 20 µM ZD7288 (B). Voltage scale is 25 mV for the original $V_m$ traces (top row) and 15 mV for the added $V_m$ traces (bottom row) in A – B. *C – E:* SDP magnitudes (C), half widths (D) and peak times (E) evoked under control conditions (gray bars) or in the presence of ZD7288 (black bars). The three measures were separated by the resting $V_m$ from which SDPs were evoked and averaged across 6 CWCs.
Ba$^{2+}$ was applied to block outward K$^+$ currents (see solutions). Additional 100 µM Ni$^{2+}$, 500 µM Cd$^{2+}$ and 100 nM TTX were added to block inward Ca$^{2+}$ and Na$^+$ currents.

The steady-state activation curve for I$_h$ was derived from tail currents (see section 2.7.). Current responses were elicited by a group of depolarizing and hyperpolarizing test voltages ranging from -150 mV to -40 mV, with steps of 5 mV, for 1.4 s (Fig. 3-7 A). Tail currents were generated while post stepped to -70 mV for 0.5 s following test steps (Fig. 3-7 A-B). Tail currents for test steps above post-step represented the activation of I$_h$, whereas those below post steps reflected its deactivation. Instantaneous tail current I$_{\text{tail}}$ ($t = 0$) saturated with deep hyperpolarizing potentials as conductance g$_h$ approached its steady-state value and decreased toward leak currents with depolarizing potentials as g$_h$ approached zero. The normalized steady-state activation curve (Fig. 3-7 C) was computed from tail currents in figure 3-7 B and fitted with a Boltzmann function (Equation 6, $V_{\text{half}} = -103.8 \pm 9.6$ mV and $K_s = 10$ mV $\pm 3.7$ mV, N = 7).

Current traces elicited by test voltage steps were replotted on an expanded scale in figure 3-7 D. The activation and deactivation of I$_h$ were best described by the sum of two exponential components while single exponential fits deviated from original current traces (data not shown). The time constants of activation $\tau_1$ and $\tau_2$ were pooled among the same 7 cells from which steady-state activation curves were derived (Fig. 3-7 E). Time constant $\tau_1$ showed a small fraction of voltage-dependence while current traces elicited by large hyperpolarizing voltage steps tend to saturate rapidly. However, when potentials went above -75 mV, $\tau_1$ became flat and showed little voltage-dependence. The reason could be that since the post-step was set at -70 mV, test potentials around that area generated little activation or deactivation of I$_h$. Thus, current traces were relatively flat,
Figure 3-7: Activation of $I_h$.  
*A*: current responses to voltage-clamp stimuli.  
*B*: tail currents from A on an expanded scale.  
*C*: steady-state activation normalized across 7 CWCs (gray shaded region).  
*D*: current responses to group of test voltage stimuli from A on an expanded scale. Biexponential fits are drawn over current traces.  
*E*: activation time constants derived from biexponential fits to current responses across 7 CWCs.  
*Upper*: $\tau_1$,  
*lower*: $\tau_2$. Error bars represent standard deviation obtained from curve fit covariance matrix.
and the exponential fits were less accurate. Time constant $\tau_2$ was much bigger than $\tau_1$ for the whole range of testing potentials. It showed the same voltage dependence as $\tau_1$ with more hyperpolarized potentials than -100 mV. Above -100 mV, errors started to increase and exponential fits had little meaningful yields.

3.5. Voltage-gated Na$^+$ currents in CWCs

3.5.1. SDPs are mediated by voltage-gated Na$^+$ current

In current-clamp mode, the voltage-gated Na$^+$ antagonist TTX (100 nM - 1 $\mu$M) was utilized to investigate the role of Na$^+$ channels in producing subthreshold responses of CWCs. A high concentration of TTX (1 $\mu$M) produced little block of SDPs (Fig. 3-8 A and C). However, stimuli near or above the AP threshold level in control solutions may activate the Ca$^{2+}$ channels that contribute to ADPs in the presence of TTX, as shown by the increased SDP magnitudes above AP threshold in TTX (Fig. 3-8 C). To prevent the activation of Ca$^{2+}$ channels from augmenting SDP magnitudes during TTX exposure, these experiments were repeated in the presence of Cd$^{2+}$ and Ni$^{2+}$ (Fig. 3-8 B and D). Although TTX did reduce SDP magnitudes in the presence of Cd$^{2+}$ and Ni$^{2+}$, a measurable component of the SDP was still present. The addition of Cd$^{2+}$ and Ni$^{2+}$ also prevented an increase in SDP magnitudes above AP threshold, confirming the contribution of Ca$^{2+}$ channels to SDP magnitudes in the presence of TTX alone. A lower concentration of TTX (100 nM) completely blocks APs (not shown), and produced a similar reduction in SDP magnitudes (Fig. 3-8 D, open circles) compared to higher concentrations (Fig. 3-8 D, filled circles). Therefore, a saturating concentration of TTX was being utilized, and a portion of the SDP appears to be TTX sensitive.
**Figure 3-8:** *SDPs are partially blocked by TTX.*  
*A:* Application of TTX (1 µM) blocks APs but does not reduce ADP magnitudes evoked by suprathreshold current injection (top two rows of $V_m$ traces). TTX also reduces SDP magnitudes evoked by subthreshold current injection (bottom two rows of $V_m$ traces).  
*B:* Responses to suprathreshold (top two rows of $V_m$ traces) and subthreshold (bottom two rows of $V_m$ traces) current injection during the application of CdCl$_2$ (100 µM) and NiCl$_2$ (500 µM) compared to responses during the application of CdCl$_2$, NiCl$_2$ and TTX.  
$V_m$ traces during the application of TTX in A and B were evoked by larger current pulses to facilitate comparison of SDP and ADP magnitudes evoked at similar $V_m$ values, as measured during the response to the depolarizing stimulus.  
*C:* SDP magnitudes vs. $V_m$ averaged across 5 CWCs under control conditions (gray shaded region) and in the presence of TTX (filled circles).  
*D:* SDP magnitudes vs. $V_m$ averaged across 5 CWCs in the presence of CdCl$_2$ and NiCl$_2$ (gray shaded region), and in the presence of CdCl$_2$ and NiCl$_2$ with 100 nM TTX (open circles) or 1 µM TTX (filled circles) added. Magnitudes of events evoked in the presence of TTX at $V_m$ greater than the original AP threshold under control conditions are included in panels C - D.
The anticonvulsants phenytoin (100 µM) and riluzole (10 µM) are known to antagonize the persistent Na\(^+\) current in cortical neurons (Lampl et al. 1998; Urbani and Belluzzi 2000), and were utilized in this study to examine the potential involvement of a persistent Na\(^+\) current in producing SDPs. Neither phenytoin nor riluzole produced any measurable reductions in SDP magnitudes (Fig. 3-9), suggesting that the voltage-gated channels that underlie the subthreshold responses of CWCs are distinct from those that are responsible for producing the subthreshold responses of cortical pyramidal neurons. Although these drugs have no observable effect on SDP magnitudes, a slight depression in the AP magnitude is observed during the application of phenytoin and riluzole (Fig. 3-9 B, open circles). Thus, a small fraction of Na\(^+\) channels responsible for APs could be blocked by these agents, whereas the Na\(^+\) channels that underlie SDP generation are unaffected.

### 3.5.2. Voltage dependence of persistent Na\(^+\) current

To further investigate if any other type of persistent Na\(^+\) current exists in CWCs, a slow voltage ramp protocol (50 mV/sec), from -110 mV to 40 mV, was applied (Fig. 3-10) to isolate persistent Na\(^+\) currents from fast inactivating Na\(^+\) currents. In control media, KCl and KH\(_2\)PO\(_4\) of ACSF were replaced by 4-AP (1 mM) and TEACl (10 mM). Strychnine (1 µM), picrotoxin (5 µM) and kynurenic acid (1 mM) were added to block ionotropic excitatory and inhibitory synaptic transmission. Cd\(^{2+}\) (100 µM) and Ni\(^{2+}\) (500 µM) were added to block voltage-gated Ca\(^{2+}\) currents; TTX (100 nM) was added to block Na\(^+\) currents. Figure 3-10 shows the inward currents elicited by the ramp voltage protocol.
Figure 3-9: Anticonvulsants phenytoin and riluzole have no effect on SDP magnitudes. A: Application of phenytoin (100 µM) and riluzole (10 µM) slightly reduce the magnitudes of APs elicited by suprathreshold stimuli (top two rows of V_m traces) but have no effect on subthreshold responses (bottom two rows of V_m traces).

B: Time course of AP (open circles) and SDP (filled circles) magnitudes during the application of phenytoin averaged across 8 CWCs and during the application of riluzole averaged across 5 CWCs. Because of their large variability across cells, AP magnitudes were normalized to those measured under control conditions prior to averaging across cells. C: SDP magnitudes vs. V_m averaged across 6 CWCs under control conditions (gray shaded region), in the presence of phenytoin (filled circles), and in the presence of riluzole (open circles).
**Figure 3-10:** Na$^+$ current elicited by a slow ramp protocol in CWCs. A: Currents evoked by ramp protocol in control solution and during the addition of TTX (100 nM) in one CWC. The ramp protocol depolarizes the membrane potential from -110 mV to 40 mV at a rate of 50 mV/sec. Traces are averaged responses to 20 - 30 ramp stimuli. B: Difference between control and TTX traces demonstrates TTX-sensitive persistent Na$^+$ currents averaged across 5 CWCs. Gray shaded region represents standard error of the mean; solid trace is resulting fit of steady-state activation function (equation 6).
in a CWC; the maximal persistent current amplitude of -212 pA was evoked at -30 mV. Following the application of TTX, the maximum inward current decreased to -15 pA at -27 mV (Fig. 3-10 A). Subtracting current traces obtained in the presence of TTX from those obtained under control conditions revealed a TTX-sensitive persistent Na\(^+\) current in CWCs (Fig. 3-10 B, N = 5). When fitted with a Boltzmann function, the subtracted traces showed the presence of a persistent Na\(^+\) current with a half-activation potential \(V_{\text{half}}\) at -50.4 mV ± 9.9 mV and an activation slope factor \(K_s\) of 4.6 mV ± 1.2 mV. This persistent Na\(^+\) current in CWCs might play an important role of generating SDPs in CWCs, since it could activate at voltage levels below the average spike threshold (-51 mV ~ -45 mV).

3.6. K\(^+\) currents in CWCs

3.6.1. Voltage-gated K\(^+\) currents in CWCs

4-aminopyridine (4-AP) sensitive K\(^+\) channels have been reported in complex-spiking hippocampal pyramidal neurons (Golding et al. 1999; Hu et al. 2001), suggesting that voltage-gated K\(^+\) channels might play a role in generating complex spikes. Therefore, 4-AP was used to investigate the role of voltage-gated K\(^+\) channels for regulation of complex spiking in CWCs. Application of 4-AP (100 µM – 1 mM) could produce complex spiking responses in simple spiking CWCs, but not in pyramidal cells (Fig. 3-11). The application of 4-AP resulted in a depolarization of the pyramidal cell by approximately 10 mV and the increases of half width of APs by around 1 msec (Fig. 3-11, left). These effects were caused by blocking voltage-gated K\(^+\) currents in pyramidal cells. However, the firing patterns of pyramidal cells remained unchanged. In contrast,
Figure 3-11: 4-AP (1 mM) produces complex spiking responses in simple spiking CWCs, but not in pyramidal cells. Left: The application of 4-AP resulted in a depolarization of the pyramidal cell by approximately 10 mV, increased the half width of APs by 1 msec. Right: The application of 4-AP did not affect the holding potential of the CWC, but converted the simple spike into a complex spike (N = 5).
the application of 4-AP did not affect the holding potential of the CWC; however, the suprathreshold responses were substantially affected as simple spike were converted into complex spikes with an initial narrow AP followed by a broad, slow AP (Fig. 3-11, right, N = 5). These results show that the inability of some CWCs to produce complex spikes may be due to the regulation of suprathreshold response patterns by K⁺ currents.

3.6.2. Ca²⁺-activated K⁺ currents in CWCs

Studies showed that Ca²⁺ channel antagonists NiCl₂ and CdCl₂ could reversibly block ADPs, confirming the contribution of Ca²⁺ currents to suprathreshold responses (Fig. 3-4 A). Further investigation revealed a role for Ca²⁺-activated K⁺ currents during the repolarization of suprathreshold responses as shown by a reversible increase in the AP half-width during application of NiCl₂ and CdCl₂ (Fig. 3-12 A). Although the rising phase of the AP was almost the same as that in control, the falling phase of the AP returned to the resting potential with a slower time course during the application of NiCl₂ and CdCl₂. This can be attributed to a blockade of Ca²⁺-activated K⁺ currents, which contributed to the repolarization of the AP along with other voltage-gated K⁺ currents. AP half-widths averaged across 9 CWCs demonstrated that the AP half-width increased from 0.79 ± 0.05 mV in control to 1.6 ± 0.07 mV during application of NiCl₂ and CdCl₂; and then returned to 1.0 ± 0.06 mV during a washout in control solution (Fig. 3-12 B).

3.7. Pre-step voltage dependence of CWCs

Previous studies (Manis et al. 1994; Zhang and Oertel 1993) and recordings in our lab have demonstrated that the Ca²⁺ currents that underlie the complex spikes and ADPs
**Figure 3-12:** Voltage-gated Ca$^{2+}$ current antagonists Ni$^{2+}$/Cd$^{2+}$ increased the half-width of APs. **A:** Addition of CdCl$_2$ (100 µM) and NiCl$_2$ (500 µM) reversibly widened the action potential (AP) in response to suprathreshold current injection. **B:** Comparisons of half-widths between control, application of CdCl$_2$ and NiCl$_2$, and washout averaged across 9 CWCs.
do not inactivate with depolarized resting membrane potentials. In contrast, our results indicate that complex spiking is facilitated by a depolarized resting membrane potential (Fig. 3-13 A-C). The complex spikes, evoked from resting potentials with depolarizing current injection (Fig. 3-13 A-B, upper panels), were converted into simple spikes with ADPs when preceded by a 100 msec pulse of hyperpolarizing current (Fig. 3-13 A-B, middle and lower panels). The amplitudes of ADPs following the initial APs were also depressed when the amplitude of the hyperpolarizing pre-step was increased (Fig. 3-13 A-B: -100 pA for middle panels; -200 pA for lower panels).

The underlying mechanism of this observation might come from two possible sources: the inactivation of outward currents contributing to the repolarization of ADPs or the facilitation of inward currents contributing to the depolarization that underlies ADPs. One candidate is \( I_h \), which is activated by hyperpolarization but contributes to the repolarization of SDPs and ADPs (Fig. 3-5, also see simulation results below). However, the phenomenon of pre-step voltage dependence could not be reproduced in a model CWC (data not shown), suggesting either CWCs might possess different kinetic properties of \( I_h \) from those used in this model, or another current is involved. Another candidate is the transient \( K^+ \) current, which is inactivated at resting potentials but deinactivated at hyperpolarized membrane potentials. However, further studies with the voltage-gated \( K^+ \) channel antagonist 4-AP did not eliminate the facilitation of complex spiking by depolarized membrane potentials (Fig. 3-13 C-D, \( N = 3 \)). In contrast, application of 0.5 mM 4-AP to CWCs converted simple spikes into repetitive complex spikes when evoked from normal resting potentials; repetitive complex spikes were
Figure 3-13: Pre-step voltage dependence of CWCs.  

A: Simple spikes are elicited when a hyperpolarizing voltage step is applied prior to the depolarization. The ADP increases in magnitude as the pre-step hyperpolarization decreases.  
B: Traces in A shown on an expanded scale to demonstrate the decrease in ADP magnitude with increased pre-step hyperpolarization.  
C-D: In a simple spiking CWC, 0.5 mM 4-AP elicits repetitive complex spiking that can be attenuated with a pre-step hyperpolarization.
Control 50 ms 20 mV 1 nA 0.5 mM 4AP

20 mV 1 nA 50 ms

DC

A  B

C  Control

D  0.5 mM 4AP

20 mV 1 nA 50 ms
converted into simple spikes followed by ADPs when evoked from hyperpolarized membrane potentials.
Chapter Four

Simulation Results

To further study the relationship between ionic properties and various firing patterns in CWCs, a single compartment model of CWCs was used to simulate responses to subthreshold and suprathreshold current injection. The results of these simulations confirmed various results that were observed experimentally. First, SDPs in model CWC were generated not by high-threshold voltage-gated Ca\(^{2+}\) current but by persistent Na\(^+\) current. Second, suprathreshold complex spikes could be converted into simple spikes when conductance of high-threshold voltage-gated Ca\(^{2+}\) current was reduced. Simple spiking responses could be converted to complex spikes when the conductance levels of any of three voltage-gated K\(^+\) currents were decreased. Finally, I_h was shown to contribute to the repolarization of simulated responses to depolarizing stimuli.

4.1. Ionic currents in model of CWCs

A model of CWC was created by inserting several Hodgkin-Huxley style models of voltage-gated ion channels into a single compartment to represent the cell body from which electrophysiologic recordings were obtained. These voltage-gated ionic currents include 4 inward currents and 3 outward currents. The inward currents are a fast Na\(^+\)
current, a persistent Na\(^+\) current, an N-type voltage-gated Ca\(^{2+}\) current and the HCN2 I\(_h\) current. The outward currents are a delayed-rectifier K\(^+\) current, a transient K\(^+\) current and a Ca\(^{2+}\)-activated K\(^+\) current.

4.1.1 Fast Na\(^+\) current (I\(_{Na}\))

The voltage-dependence and kinetics of the fast Na\(^+\) current in DCN CWCs has not been investigated up to the present time. Therefore, a model of Na\(^+\) currents from cortical pyramidal neurons (Bernander et al. 1994) was implemented in the present study (Fig. 4-1). Using a Hodgkin-Huxley style of formalism, the kinetic properties of the fast Na\(^+\) current are modeled using the following equations:

\[
I_{Na}(V, t) = G_{Na} m_{Na}^2 h_{Na} (V - V_{Na})
\]

(7)

\[
m_{Na\_inf}(V) = (1 + e^{-(V + 38)/3})^{-1}
\]

(8)

\[
h_{Na\_inf}(V) = (1 + e^{(V + 43)/3})^{-1}
\]

(9)

The activation and inactivation time constants were set voltage independent constants 0.5 msec and 0.05 msec. The maximal conductance of fast Na\(^+\) current, \(G_{Na}\), was set as 28.57 mS/cm\(^2\) to produce a model with similar firing patterns as those obtained from experimental recordings. Some aspects of suprathreshold responses of CWCs, such as AP threshold, AP width or spike intervals, are affected by changing the maximal conductance of the fast Na\(^+\) current. However, changing the maximal conductance of the fast Na\(^+\) current did not have measurable effects on the magnitude and time course of SDPs, ADPs and complex spikes, therefore this value was maintained at a constant value for the purposes of this modeling study.
Figure 4-1: *Kinetic properties of Fast Na⁺ current.* m: steady-state activation. h: steady-state inactivation. \( \tau_m \): activation time constant, 0.5 msec. \( \tau_h \): inactivation time constant: 0.05 msec.
4.1.2. Persistent Na\(^+\) current (I\(_{\text{NaP}}\))

Whole-cell voltage clamp recordings revealed that a persistent sodium current was present in CWCs and activated at membrane potentials below spike threshold. This result leads to the hypothesis that a persistent sodium current is the candidate to generate SDPs in CWCs, and may play a role contributing to the ability of CWCs to produce complex spikes in response to stimuli just above spike threshold. The steady-state activation curve of I\(_{\text{NaP}}\) in this model was directly derived from experimental data (Fig. 4-2). The half-activation potential was set as \(-50.4\) mV and slope factor as 4.6 mV. The kinetic equations are as follows,

\[
I_{\text{NaP}}(V,t) = G_{\text{NaP}}m_{\text{NaP}}(V - V_{\text{Na}}) \quad (10)
\]

\[
m_{\text{NaP\_inf}}(V) = (1 + e^{-(V+50.4)/4.6})^{-1} \quad (11)
\]

Because of the inability to pharmacologically separate persistent and transient Na\(^+\) currents, the activation time constants of persistent sodium currents in CWCs have not been measured experimentally. The value used in this model was obtained from searching other existing persistent models for close activation steady-state curves as the one showed in this thesis study. A voltage-dependent time constant for activation was then chosen from a persistent sodium current model in cerebellar Purkinje cells (De Schutter and Bower 1994a, b). The kinetic equation is as follows:

\[
\tau_{m\_\text{NaP}}(V) = \left[ \frac{200}{1 + e^{-(V-16)/16}} + \frac{25}{1 + e^{(V+58)/8}} \right]^{-1} \quad (12)
\]

The maximal conductance of persistent sodium current, \(G_{\text{NaP}}\), was set as 0.05 mS/cm\(^2\), which was originally estimated from physiological values and then adapted in this model to obtain firing patterns close to experimental results.
**Figure 4-2:** Kinetic properties of persistent $Na^+$ current. m: steady-state activation.

$\tau_m$: time constant of activation (ordinate on the right).
4.1.3. N-type voltage-gated Ca\(^{2+}\) current (I\(_{\text{CaN}}\))

Specific subtypes of voltage-gated Ca\(^{2+}\) currents in DCN CWCs were not studied in this thesis. Previous studies on isolated DCN neurons suggested that high-threshold voltage-gated N-type Ca\(^{2+}\) channels were likely to be present in CWCs, although a complete model of these currents was not developed during pharmacologic isolation (Molitor and Manis 1999). A model of N-type voltage-gated Ca\(^{2+}\) current from hippocampal pyramidal neurons was implemented (Jaffe et al. 1994) with similar voltage- and time-dependence to the Ca\(^{2+}\) currents from isolated DCN neurons (Fig. 4-3).

The kinetic equation is presented as follows:

$$I_{\text{CaN}}(V,t) = -G_{\text{CaN}} m_{\text{GaN}}^2 h_{\text{GaN}} V \frac{1 - \left(\frac{[\text{Ca}^{2+}]_0}{[\text{Ca}^{2+}]_0}ight) e^{2FV/kT}}{1 - \left(1 - \frac{[\text{Ca}^{2+}]_0}{[\text{Ca}^{2+}]_0}\right) e^{2FV/kT}}$$ (13)

where \(F\) is Faraday’s constant, \(k\) is Boltzmann’s constant and \(T\) is temperature.

The transitions of activation and inactivation gates between open (s) and closed (1-s) positions are described as a first-order reaction:

$$\begin{align*}
\alpha & \\
s & \leftrightarrow 1 - s \\
\beta &
\end{align*}$$

for \(s = m, h\) (14)

\(\alpha\) is the forward rate constant and \(\beta\) the backward rate constant. Thus, steady-state of activation and inactivation, as well as time constants, could be calculated by using these two rate constants:

$$s = \frac{\beta}{\alpha + \beta} \quad \text{for } s = m, h$$ (15)

$$\tau_s = \frac{1}{\alpha + \beta} \quad \text{for } s = m, h$$ (16)
Figure 4-3: Kinetic properties of N-type voltage-gated Ca\(^{2+}\) current.  

A: m as steady-state activation; \(\tau_m\) as time constant of activation (ordinate on the right).  

B: h as steady-state inactivation; \(\tau_h\) as time constant of inactivation (ordinate on the right).
Rate constants $\alpha$ and $\beta$ for both $m$ and $h$ are voltage-dependent, which are

$$\alpha_m = 0.197 \frac{(19.88 - V)}{e^{(19.88 - V)/10} - 1}$$  \hspace{1cm} (17)$$

$$\beta_m = 0.046e^{(-V/20.73)}$$  \hspace{1cm} (18)$$

$$\alpha_h = 1.6 \times 10^{-4} e^{(-V/48.4)}$$  \hspace{1cm} (19)$$

$$\beta_h = \frac{1}{1 + e^{(39-V)/10}}$$  \hspace{1cm} (20)$$

The driving force was determined by the Goldman-Hodgkin-Katz equation presented in Equation 13. $[Ca^{2+}]_i$ is intracellular concentration of Ca$^{2+}$, which was set as NEURON default value, 50 nM. $[Ca^{2+}]_o$ is extracellular concentration of Ca$^{2+}$, which was set as the Ca$^{2+}$ concentration of ACSF, 2.5 mM. The default conductance value for the N-type Ca$^{2+}$ current was adjusted to 2 mS/cm$^2$ to produce suprathreshold responses similar to those from experimental results.

4.1.4. Hyperpolarization-activated $h$ current ($I_h$)

Experiments on whole-cell voltage-clamp recordings suggested the presence of HCN2 $I_h$ current in CWCs (see discussion). A previously presented $I_h$ model of thalamic relay neurons (Destexhe and Babloyantz 1993; Destexhe et al. 1993) was adopted in this study with modified properties to better describe the currents obtained during voltage clamp recordings from CWCs (section 3.4.2). The equations for this current are as follows,

$$I_h(V, t) = G_h m_h n_h (V - V_h)$$ \hspace{1cm} (21)$$

$$m_{h_{\text{inf}}} = n_{h_{\text{inf}}} = \frac{1}{1 + e^{(V+68.9)/6.5}}$$ \hspace{1cm} (22)$$
**Figure 4-4:** Kinetic properties of $I_h$. 

**A:** $m$ as steady-state of first activation; $\tau_m$ as time constant of first activation (ordinate on the right). 

**B:** $n$ as steady-state of second activation; $\tau_n$ as time constant of second activation (ordinate on the right).
\[ \tau_m = 100 + e^{(V+183.6)/15.24} \]  

(23)

\[ \tau_n = \frac{1 + e^{(V+158.6)/11.2}}{1 + e^{(V+75)/5.5}} \]  

(24)

Two activation processes are used since voltage-clamp recordings showed that \( I_h \) from voltage-clamped CWCs was fit more accurately by two exponential components (Fig. 3-7 D). The steady-state curves of two activations are identical, and the half-activation potential and slope factor are kept unchanged from the original model (\( V_{\text{half}} = -68.9 \) mV; \( K_s = 6.5 \) mV). These values are not adopted from experimental recordings (\( V_{\text{half}} = -103.8 \) mV; \( K_s = 10 \) mV) based on the hypothesis that during the voltage-clamp recordings in this study, \( I_h \) currents activated by test potentials possessed attenuated amplitudes and did not reach the steady states at the time when tail currents were elicited, possibly causing the inaccuracy of obtaining kinetic properties of steady-state activations (see discussion). The time constant of first activation is increased by a constant value of 100 msec to make it close to the one obtained from experimental recordings. Maximal conductance \( G_h \) was set as 0.25 mS/cm\(^2\) for same purpose.

4.1.5. Delayed-rectifier K\(^+\) current (\( I_{dr} \))

The voltage-dependence and kinetics of the delayed-rectifier K\(^+\) current in DCN CWCs has not been investigated at the present time. Therefore, a model of noninactivating K\(^+\) current from study on DCN PC (Kanold and Manis 1999) was implemented in the present study (Fig. 4-5). The maximal conductance is changed from 6.7 mS/cm\(^2\) to 2 mS/cm\(^2\) to better describe the firing patterns in CWCs. The kinetic equations are as follows:
Figure 4-5: Kinetic properties of delayed-rectifier $K^+$ current. $m$ as steady-state activation; $\tau_m$ as time constant of activation (ordinate on the right).
\[ I_{dr}(V,t) = G_{dr} m_{dr}^2 (V - V_k) \]  
\[ m_{dr \text{ inf}} = \frac{1}{1 + e^{-(V+43)/3}} \]

The activation time constant was set to 0.5 msec.

4.1.6. Transient K$^+$ current ($I_A$)

Experimental results showed that 1 mM 4-AP extracellularly could convert simple-spiking CWCs to complex-spiking ones, suggesting the regulatory role of K$^+$ channels in CWCs. Previous study had suggested that the inactivating K$^+$ currents in DCN CWCs may have similar kinetic properties as transient K$^+$ currents in DCN PCs (Molitor, unpublished). Thus, the kinetic equations are adopted from the study of slow-inactivating K$^+$ channels in DCN pyramidal neurons (Kanold and Manis 1999) (Fig. 4-6).

\[ I_A(V,t) = G_A m_A^4 h_A (V - V_k) \]  
\[ m_A \text{ inf} = \frac{1}{1 + e^{-(V+40.9)/23.7}} \]  
\[ h_A \text{ inf} = \frac{1}{1 + e^{-(V+38.4)/9}} \]

\[ \tau_m = 0.5 + \frac{1}{0.15e^{(V+40)/10} + 0.3e^{-(V+40)/10}} \]

The inactivation time constant was set as 200 msec and the maximal conductance was set as 2 mS/cm$^2$ to obtain firing patterns close to experimental results.
Figure 4-6: Kinetic properties of transient $K^+$ current. 

**A**: m as steady-state activation; $\tau_m$ as time constant of activation (ordinate on the right). 

**B**: h as steady-state inactivation; $\tau_h$ as time constant of inactivation, which is constant 200 msec (ordinate on the right).
4.1.7. Ca\(^{2+}\)-activated K\(^+\) current (I\(_{KCa}\))

Experimental results showed that the voltage-gated Ca\(^{2+}\) channel antagonists NiCl\(_2\) and CdCl\(_2\) increased the width of action potentials (Fig. 3-13), suggesting the presence of Ca\(^{2+}\)-activated K\(^+\) currents in CWCs. A modified Ca\(^{2+}\)-activated K\(^+\) current model was implemented in this study (Moczydlowski and Latorre 1983) to account for this current (Fig. 4-7).

\[
I_{KCa}(V,t) = G_{KCa}m_{KCa}(V-V_K)
\]  
\[\alpha\]
\[m_{KCa} \leftrightarrow 1 - m_{KCa} \]
\[\beta\]

For the activation gate m\(_{KCa}\), \(\alpha\) is the forward rate constant and \(\beta\) the backward rate constant. Thus, the steady-state activation m\(_{KCa}\) (\(t = \infty\)) and time constant \(\tau_m\) could be calculated by using these two rate constants:

\[
m_{KCa_{\infty}} = \frac{\beta}{\alpha + \beta}
\]  
\[\tau_m = \frac{1}{\alpha + \beta}
\]  
\[
\alpha = \frac{1.4 \cdot [Ca^{2+}]_i}{[Ca^{2+}]_i + 0.48 \times 10^{-3} e^{-1.68FV/\kappa T}}
\]  
\[\beta = \frac{2.4}{1 + 0.13 \times 10^{-6} \cdot e^{-2FV/\kappa T}}
\]

The maximal conductance \(G_{KCa}\) was set as 0.55 mS/cm\(^2\) to obtain firing patterns close to experimental results.
Figure 4-7: Kinetic properties of Ca$^{2+}$-activated K$^+$ current. m as steady-state activation; $\tau$ as time constant of activation (ordinate on the right). Both m and $\tau$ are dependent on intracellular Ca$^{2+}$ concentration, [Ca$^{2+}$]$_i$. Black lines of m and $\tau$: [Ca$^{2+}$]$_i$ = 0.1 mM; dashed lines of m and $\tau$: [Ca$^{2+}$]$_i$ = 0.01 mM; gray lines of m and $\tau$: [Ca$^{2+}$]$_i$ = 0.001 mM.
4.2. Complex-spiking CWC model

The electrophysiological responses of a single compartment model of CWCs were compared with responses obtained experimentally using 100 msec pulses of depolarizing current injection (Fig. 4-8). Just-above threshold stimuli generated a 2-AP burst (Fig. 4-8, upper left) and well-above-threshold stimuli elicited a train of APs following the 2-AP burst (Fig. 4-8, lower left) in a real cell. Similarly, the model responded to just-above threshold somatic injection with a 2-AP burst (Fig. 4-8, upper right), as well as similar train of complex spikes when the amplitude of the injection increased (Fig. 4-8, lower right). In both cases, the amplitude of second AP in each burst was smaller than the first AP, indicating the partial recovery of fast Na\textsuperscript{+} currents from inactivation. When presented with hyperpolarizing stimuli, both cells showed ‘sag’ depolarizations, which are characteristic responses of neurons will substantial I\textsubscript{h} currents. The half widths of APs in the real cell were 0.8 msec compared with 1.1 msec in the model. Despite these similarities, the model had a larger input resistance, responded to suprathreshold stimuli with larger APs, and exhibited faster repolarization following the second AP in the burst. These differences may be attributed to inaccurate kinetics of the fast Na\textsuperscript{+} current used in the CWC model, or because of a lack of dendritic processes in the compartmental model. Regardless, the model possessed many similarities, particularly with respect to the SDPs, ADPs and complex spikes that were characteristic of CWCs during whole cell recordings.
**Figure 4-8:** Comparison between experimental and modeling results. Left panel shows the whole-cell recordings from a CWC which was held at -65 mV. Right panel is the whole-cell simulation from the model which was held at -63.4 mV. *Upper:* Just-above threshold stimuli elicit complex spikes both in real and model neuron. *Lower:* Well-above threshold stimuli elicit complex spikes followed by train of simple spikes both in real and model neuron.
4.3. Persistent Na\(^+\) current contributes to SDPs

A brief stimuli (2 msec) current clamp protocol similar to the one used in electrophysiological experiments was applied to investigate the role of persistent Na\(^+\) current (I\(_{\text{NaP}}\)) in regulating the firing patterns of CWCs (Fig. 4-9). In electrophysiological experiments, it was not possible to distinguish between the persistent Na\(^+\) current and the fast Na\(^+\) current pharmacologically because both currents were blocked by 100 nM TTX, and the persistent Na\(^+\) current in CWCs showed resistance to other Na\(^+\) channel antagonists such as riluzole and phenytoin. However, the computational model of CWCs provides a method to examine the role of persistent Na\(^+\) currents in CWCs.

Suprathreshold responses (Fig. 4-9 A, upper) and subthreshold responses (Fig. 4-9 A, middle) were evoked from resting potentials. In response to subthreshold stimuli under normal situations, SDPs were evoked by subthreshold depolarization in the model that were similar in amplitude and time course when compared to those observed experimentally (Fig. 4-9 A, Middle, gray line). However, when the persistent Na\(^+\) current was removed, the model responded with only a passive membrane potential decay, which clearly deviated from the SDPs observed under normal conditions, revealing that persistent Na\(^+\) currents are required to evoke SDPs in the model. The time course of ionic currents confirmed that I\(_{\text{NaP}}\) was contributing to SDP (Fig. 4-9 A, Lower). In addition to the effects on subthreshold responses, simulated responses also demonstrated that the persistent Na\(^+\) current could modulate the suprathreshold firing pattern of CWC (Fig. 4-9 A, Upper). Under normal conditions, the model responded to suprathreshold depolarization with complex spikes, whereas the model responded with simple spikes.
Figure 4-9: Persistent Na$^+$ current ($I_{NaP}$) in CWCs. A: Suprathreshold and subthreshold responses were affected by the persistent Na$^+$ current. Upper: Suprathreshold responses of the model with or without the persistent Na$^+$ current. The complex spiking model was converted into a simple spiking model when the maximal conductance of persistent Na$^+$ current, $G_{NaP}$, was set to be 0% of its original value. Middle: Subthreshold responses of the model with or without the persistent Na$^+$ current. SDP was decreased dramatically when $G_{NaP}$ was set to be 0% $G_{NaP}$. Lower: Time course of $I_{NaP}$ responding to subthreshold stimuli. B: SDP (circles) and ADP (open triangles) amplitudes as a function of maximal $G_{NaP}$ conductance level. Filled triangles represent complex spikes. 100% $G_{NaP}$ represents the control value used in the model. SDPs increase as $G_{NaP}$ increases and saturate around 150% $G_{NaP}$. Complex spikes were converted into simple spikes when $G_{NaP}$ was below 80% of control value. C: Increasing the amplitude of stimuli by three times (black line) compared to control levels (gray line) could not evoke a complex spike when the persistent Na$^+$ current was removed from the model. D: Increasing the duration of stimuli by three times (black line) compared to control durations (gray lines) changes the suprathreshold responses from a simple spike to a complex spike in the absence of the persistent Na$^+$ current.
A

B

C

D

10 ms

20 mV

2 nA

10 ms

20 mV

4 nA

Percentage of G_NaP (%)

SDP

ADP

complex spike

mV

0 20 50 100 120 150 200

0 2 4 6 8 10 11

G_{NaP=0}
followed by a smaller ADP when the persistent Na\(^+\) current was removed. Without I\(_{\text{NaP}}\), complex spikes were not elicited even after increasing the amplitude of the brief stimuli (Fig. 4-9 C), although increasing the duration of the stimulus could recover complex spiking by activating high-threshold Ca\(^{2+}\) currents (Fig. 4-9 D). Simulations utilizing a range of maximal conductance values of the persistent Na\(^+\) current (G\(_{\text{NaP}}\)) showed that the magnitude of SDPs just below AP threshold increases as G\(_{\text{NaP}}\) increases from no persistent current (0% of control value) to 150% of control value and saturated above that (Fig. 4-9 B, circles); ADPs just above AP threshold increase as G\(_{\text{NaP}}\) increases from 0% to 80% of control value, and then are converted into complex spikes when G\(_{\text{NaP}}\) goes above 80% of control value (Fig. 4-9 B, triangles). These results suggested that the persistent Na\(^+\) current not only underlies the responses of CWCs to subthreshold depolarization; but also contributes to the generation of complex spikes in response to suprathreshold depolarization.

### 4.4. Variable activation threshold of persistent Na\(^+\) currents

Voltage-clamping recordings on the persistent Na\(^+\) currents in CWCs revealed a steady-state activation curve with mean half-activation potential (V\(_{\text{half}}\)) of -50.4 mV (Fig. 3-10). We found that V\(_{\text{half}}\) varied from -40 mV to -60 mV across examined CWCs, leading to the hypothesis that, besides the change in maximal conductances, various steady-state activation curves of persistent Na\(^+\) current could also affect the firing patterns in CWCs. Further study with the model supported this hypothesis. When V\(_{\text{half}}\) of the persistent Na\(^+\) current steady-state activation curve was shifted positively from -50.4 mV to -40.4 mV, suprathreshold responses were converted from a complex spike to a
Figure: 4-10: Persistent Na⁺ current affects suprathreshold responses in CWCs by changing $V_{\text{half}}$ of steady-state activation curve. A: Steady-state activation curves of persistent Na⁺ currents with control $V_{\text{half}}$ value (gray: $V_{\text{half}} = -50.4$ mV) vs. depolarized $V_{\text{half}}$ value (black: $V_{\text{half}} = -40.4$ mV). Inset: model responded to suprathreshold stimuli with a complex spike under control conditions, and a simple spike followed by an ADP with depolarized $V_{\text{half}}$ value. B-C: time courses of persistent Na⁺ currents ($I_{\text{NaP}}$ of dashed line) and high-threshold Ca²⁺ currents ($I_{\text{Ca}}$ of solid line) with $V_{\text{half}}$ of persistent Na⁺ currents at -40.4 mV in B or -50.4 mV in C. Whereas the activation of Ca²⁺ currents was almost identical during and after the first AP of the complex spike, the amplitude of the persistent Na⁺ current with $V_{\text{half}} = -50.4$ mV is larger than that with $V_{\text{half}} = -40.4$ mV (arrows), causing a further depolarization that triggers the second AP of complex spike. The amplitudes of persistent Na⁺ currents are magnified 10X for direct comparison to Ca²⁺ currents. Membrane potential traces from the inset of A shown at the top of the graphs provide the time course of a complex spike in C and a simple spike in B for comparison. D: Steady-state activation curves of the persistent Na⁺ currents with control $V_{\text{half}}$ value (gray: $V_{\text{half}} = -50.4$ mV) and hyperpolarized $V_{\text{half}}$ value (black: $V_{\text{half}} = -55.4$ mV). Inset: model responded to subthreshold stimuli with a SDP under control condition and a prolonged SDP under hyperpolarized $V_{\text{half}}$ value. E: SDP (circles) and ADP (open triangles) amplitudes as a function of $V_{\text{half}}$ value of the persistent Na⁺ current. Filled triangles represent complex spikes. The SDP amplitudes increase dramatically as $V_{\text{half}}$ shifts to hyperpolarized levels. Complex spikes were converted into simple spikes when $V_{\text{half}}$ shifts to depolarized levels.
simple spike followed by an ADP (Fig. 4-10 A, inset). Examination of time courses of ionic currents revealed that, when $V_{\text{half}}$ was shifted from -50.4 mV to -40.4 mV, the amplitude of the persistent Na$^+$ current following the repolarization of the first AP was decreased due to less activation (arrows on Fig. 4-10 B-C). Although the amplitudes and durations of the high-threshold Ca$^{2+}$ currents were kept unchanged, the membrane potential was not depolarized sufficiently by persistent Na$^+$ currents to elicit the second AP spike of the burst. When $V_{\text{half}}$ of the persistent Na$^+$ current steady-state activation curve was shifted negatively from -50.4 mV to -55.4 mV, the suprathreshold responses remained unchanged, but the subthreshold responses were substantially prolonged and exhibited a slow return to resting membrane potentials following the offset of the depolarizing stimulus. When $V_{\text{half}}$ was shifted to -60.4 mV, the amplitude of the SDP was substantially increased, revealing the strong activation of persistent Na$^+$ current below the AP threshold (Fig. 4-10 D-E). Amplitudes of SDPs and ADPs with $V_{\text{half}}$ ranging from -60.4 mV to -40.4 mV demonstrated that depolarized $V_{\text{half}}$ values affected suprathreshold responses by converting complex spikes into simple spikes; whereas hyperpolarized $V_{\text{half}}$ values affected subthreshold responses by increasing the amplitudes of SDPs. These results suggest that variations in the steady-state activation of persistent Na$^+$ currents may play a differential role in regulating the suprathreshold and subthreshold responses of CWCs.

4.5. N-type voltage-gated Ca$^{2+}$ current and suprathreshold responses

Responses to suprathreshold stimuli with various maximal conductances of the N-type voltage-gated Ca$^{2+}$ currents confirmed its role in the generation of complex spikes in
CWCs (Fig. 4-11). Under normal conditions, the model responded to suprathreshold stimuli with complex spikes (Fig. 4-11 A, upper, gray line). When the conductance of N-type voltage-gated Ca\(^{2+}\) current (\(G_{\text{CaN}}\)) was reduced to 10% or 90% of control levels, the suprathreshold response of the model was converted from a complex spike to simple spike without ADP (Fig. 4-11, upper, black line); or a simple spike followed by an ADP (Fig. 4-11, upper, dashed line). In addition to the change in firing pattern, the width of ADP was reduced when compared to the magnitude of the slow underlying depolarization during complex spiking under the control condition, indicating that the reduced conductance of N-type voltage-gated Ca\(^{2+}\) current could not provide a sustained depolarization sufficient to generate a second spike in the AP burst (Fig. 4-11 B, triangles). Conversely, simple spikes could be converted into complex spikes with multiple bursts when \(G_{\text{CaN}}\) was increased (Fig. 4-11 B, filled triangles). Despite the change in firing patterns with suprathreshold stimuli, the conductance change of N-type voltage-gated Ca\(^{2+}\) current had little effect on subthreshold responses (Fig. 4-11 A, lower). In all tested conditions (10%, 50%, 90%, 100%, 150% and 200% \(G_{\text{CaN}}\)), SDP amplitudes were unchanged (Fig. 4-11 B, circles), revealing that N-type voltage-gated Ca\(^{2+}\) currents activated at voltage levels more depolarized than would be required for currents to contribute to subthreshold responses.

### 4.6. Transient voltage-gated K\(^+\) currents repolarize suprathreshold responses

The modulatory role of transient voltage-gated K\(^+\) currents was investigated by adjusting the maximal conductances (\(G_{\text{K}}\)) in the model. Under control conditions, the model responded to suprathreshold stimuli with a complex spike. Increasing the maximal
Figure 4-11: N-type voltage-gated Ca$^{2+}$ current modulates suprathreshold responses in CWC model. A: Changing the maximal conductance of the N-type voltage-gated Ca$^{2+}$ current (G$_{CaN}$) produces various suprathreshold responses but almost identical subthreshold responses. Upper: Suprathreshold responses of CWC with control conductance (gray), 90% conductance (dotted line) and 10% conductance (black). Lower: Subthreshold responses of CWC. (All three traces are identical). B: SDP (circles) and ADP (open triangles) amplitudes as a function of maximal G$_{CaN}$ conductance level. Filled triangles represent complex spikes with number of spike bursts. SDPs are almost unchanged across entire range of G$_{CaN}$. ADPs increase as G$_{CaN}$ increases from 10% to 50% and are converted to complex spikes with increasing number of spike bursts when G$_{CaN}$ increases from 100% to 200%.
A

Control
10% G_CaN
90% G_CaN

10 mV
10 ms

B

SDP
ADP

n complex spike (n = number of spike bursts)

Percentage of G_CaN (%)
conductance of the transient $K^+$ current by 20% ($120\% G_{KA}$) converted the suprathreshold response from a complex spike to a simple spike followed by an ADP (Fig. 4-12 A). Examination on amplitudes of SDPs and ADPs over a range of $G_{KA}$ values (from 20% to 200% of control) demonstrated that suprathreshold responses were complex spikes with multiple bursts when $G_{KA}$ was decreased, and converted into simple spikes with decreased ADP amplitudes when $G_{KA}$ was increased (Fig. 4-12 B, triangles). However, the magnitudes of SDPs just below AP thresholds did not show a large dependence upon the value of $G_{KA}$ (Fig. 4-12 B, circles). Other voltage-gated $K^+$ currents, including delayed-rectifier and $Ca^{2+}$-activated $K^+$ currents, showed similar effects upon the subthreshold and suprathreshold responses of CWCs: complex spikes with multiple APs were evoked when the maximal conductances of $K^+$ currents were reduced; and simple spikes followed by ADPs when the maximal conductances of $K^+$ currents were increased. Similar to the transient $K^+$ current, changes in the maximal conductances of other $K^+$ currents did not have a large impact on SDPs. These results showed that voltage-gated $K^+$ currents contributed mostly to the repolarizing phase above AP threshold and had less influence on subthreshold responses.

4.7. Hyperpolarization-activated inward current ($I_h$) repolarizes subthreshold responses

Experimental results showed that $I_h$ enhanced the SDPs in CWCs, suggesting that $I_h$ in CWCs contributes to the repolarization of subthreshold responses. The simulation results confirmed this hypothesis (Fig. 4-13). The observation of ionic currents in response to subthreshold stimuli revealed that under control conditions, $I_h$ was open at
Figure 4-12: Transient $K^+$ current contributes to the repolarization of suprathreshold responses in CWCs.  

A: Suprathreshold responses with control (gray) and with increased maximal conductance of the transient $K^+$ current (120% $G_{KA}$).

B: SDP (circles) and ADP (open triangles) amplitudes as a function of maximal $G_{KA}$ conductance level. Filled triangles represent complex spikes with number of spike bursts. SDPs show little dependence on change of $G_{KA}$. ADPs decrease as $G_{KA}$ increases from 120% to 200% and are converted into complex spikes with increasing number of spike bursts when $G_{KA}$ decreases from 100% to 20%.
A

Control

120% G \text{KA}

10 mV

10 ms

B

\( n \) = number of spike bursts

\( \text{SDP} \)

\( \text{ADP} \)

\( \text{complex spike} \)
Figure 4-13: $I_h$ contributes to repolarization of SDPs. **A:** Change in firing patterns when $I_h$ was removed. The maximal conductance of $I_h$ was set as 0% of control value to represent removal of $I_h$ ($0\% G_h$). *Upper:* Suprathreshold responses. *Middle:* Subthreshold responses. *Lower:* ionic current responses to subthreshold stimuli in control. With $0\% G_h$, holding currents were increased to keep resting potential identical to those in control. **B:** SDP (circles) and ADP (open triangles) amplitudes as a function of maximal $G_h$ conductance level. When $G_h$ decreases, amplitudes of SDPs increase. However, values were very close when change of $G_h$ was small ($80\%, 100\%$ and $120\% G_h$).
A

Control

0% \( G_h \)

10 mV
1 pA
10 ms

\( I_{\text{NaP}} \)

\( I_h \)

B

\[ \text{SDP} \]

\[ \Delta \] \( n \) complex spike

(\( n = \) number of spike bursts)

\[
\begin{array}{c}
7 \\
6 \\
5 \\
4 \\
3 \\
0 \\
20 \\
50 \\
80 \\
100 \\
120 \\
150 \\
200 \\
\end{array}
\]

\[
\begin{array}{c}
7 \\
6 \\
5 \\
4 \\
3 \\
0 \\
20 \\
50 \\
80 \\
100 \\
120 \\
150 \\
200 \\
\end{array}
\]

Percentage of \( G_h \)(%)
resting potentials, which resulted in a tonic depolarization that was presumably offset by
the activation of K⁺ currents in this voltage range. When a subthreshold stimulus
activated the persistent Na⁺ current and evoked an SDP, Iₘ started to decrease during the
depolarizing phase of SDP reducing the inward current to offset outward K⁺ currents,
which resulted in the repolarizing phase of SDP (Fig. 4-13 A, Lower). When Iₘ was
removed from model (0% Gₘ), the SDP time course was extended with less repolarization
compared to control conditions (Fig. 4-13 A, Middle, black line). Similar to experimental
results observed during the application of ZD7288, the amplitudes of SDPs were
increased by reducing Gₘ, although simulation results showed that small changes in Gₘ
did not have a substantial effect (Fig. 4-13 B). In contrast to its influence on subthreshold
responses, Iₘ had little effect on suprathreshold responses as complex spikes were still
evoked when Iₘ was removed. However, the absence of Iₘ did have a similar effect on the
repolarization of suprathreshold responses, which were prolonged following the offset of
the complex spike (Fig. 4-13 A, Upper).
Chapter Five

Discussion

CWCs respond to suprathreshold stimuli with simple spikes, complex spikes or a combination of simple and complex spikes; the distinct electrophysiologic responses can have a profound impact on the output of the DCN. Until now, the biophysical mechanisms that underlie the complex electrophysiologic responses of these neurons have not been thoroughly investigated. Our results demonstrate that CWCs possess a persistent Na\(^+\) current, which is responsible for the generation of a subthreshold depolarization and facilitates the activation of high threshold Ca\(^{2+}\) currents during the suprathreshold responses in CWCs. CWCs also possess the hyperpolarization-activated inward current I\(_h\) with similar kinetic properties to the HCN2 subtype that contributes to the repolarization of subthreshold responses. Experimental and simulation results suggest that the various suprathreshold responses of CWCs are regulated by voltage-gated K\(^+\) currents present in these neurons.

5.1. Persistent Na\(^+\) current in CWCs

The present study showed that CWCs possess a persistent Na\(^+\) current (I\(_{NaP}\)). In various neuronal populations, the activation of persistent Na\(^+\) currents is associated with
many firing patterns, such as subthreshold oscillations in the membrane potential
(Agrawal et al. 2001; Dickson et al. 2000b; Magistretti and Alonso 1999; Pape and
Driesang 1998; Reboreda et al. 2003); intrinsic spontaneous firing that is independent of
synaptic input (Bevan and Wilson 1999; Kononenko et al. 2004; Mao et al. 2001;
Mathieson and Maler 1988; Nam and Hockberger 1997; Pennartz et al. 1997; Shuai et al.
2003; Taddese and Bean 2002; Takakusaki and Kitai 1997) and generation of ADPs in
bursting neurons (Azouz et al. 1996; Yue et al. 2005).

However, $I_{NaP}$ in CWCs may exhibit different kinetics. First, subthreshold
oscillations have not been observed in recordings from CWCs. Second, CWCs do not
appear to fire spontaneously during extracellular recordings from rat brainstem slices in
the absence of excitatory synaptic input (Waller et al. 1996b), and exhibit lower
spontaneous firing rates compared to other neuronal populations during in vivo
recordings from decerebrate cat DCN (Parham and Kim 1995b). Third, due to the
inability to separate $I_{NaP}$ from fast-inactivating $Na^+$ currents pharmacologically, no direct
evidence exists to demonstrate that $I_{NaP}$ is required to generate ADPs or slow
depolarizations underlying complex spikes.

Results from the present study demonstrate that $I_{NaP}$ in CWCs activates in
response to subthreshold stimulation and elicits a sustained depolarization, upon which
the activation of fast-inactivated $Na^+$ currents and high-threshold $Ca^{2+}$ currents elicit
complex spikes in response to suprathreshold stimuli. Strong activation of $I_{NaP}$ would
facilitate complex spikes by increasing the number of spike bursts whereas insufficient
activation of $I_{NaP}$ would convert complex spikes into simple spikes followed by ADPs
(Fig. 4-9, 4-10). This is consistent with the similar facilitation role for $Na^+$ channels
during complex spiking in cortical PCs (Alonso and Llinás 1989; Brumberg et al. 2000; Franceschetti et al. 1995; Mantegazza et al. 1998; Silva et al. 1991), suggesting regulation of suprathreshold responses by subthreshold activation of I_{NaP}.

Although the present study does not address the molecular identity of the Na\(^+\) channels that underlie SDP generation, no characteristic AP bursts were observed during extracellular recordings in DCN slices obtained from Nav1.6 mutant mice (Chen et al. 1999), which supports a role for Na\(^+\) channels in the generation of complex spikes. A reduction in subthreshold Na\(^+\) current was observed during whole-cell recordings in cerebellar Purkinje cells from Nav1.6 mutant mice (Raman et al. 1997), reducing the duration of complex spikes evoked by brief current pulses in these cells. The similarities between CWCs and cerebellar Purkinje neurons (Oertel and Young 2004) further support the hypothesis that Nav1.6 channels play a role in various firing patterns in CWCs.

5.2. I\(_h\) in CWCs

There are currently four identified mammalian gene types for the hyperpolarization activated inward current I\(_h\), named as HCN1-4, having various activation kinetics, voltage dependence and sensitivity to cAMP modulation (Ishii et al. 1999; Ludwig et al. 1998; Santoro and Baram 2003; Santoro et al. 1997; Santoro et al. 1998; Santoro and Tibbs 1999). With respect to activation kinetics, HCN1 activates within tens of milliseconds, HCN2 within hundreds of milliseconds, and HCN3 and HCN4 activates within seconds (Moosmang et al. 2001; Santoro and Baram 2003; Santoro et al. 2000; Santoro and Tibbs 1999). Voltage-clamp recordings in the present study demonstrate that I\(_h\) in CWCs has an activation time constant \(\tau_1\) above 100
milliseconds (Fig. 3-7 E), suggesting the presence of HCN2 in CWCs. This finding is supported by a previous immunohistochemical study in young rats showing that HCN2 but not HCN1 was found in the DCN (Koch et al. 2004). Although a steady-state activation curve of $I_h$ was obtained in the present study, the kinetic properties, half-activation potential of -103.6 mV and slope factor of 10 mV were not used in the model because of two reasons. First, the inward currents responding to hyperpolarizing test steps in Figure 3-7 D may not reach steady-state values before tail currents were elicited when the duration of test steps used in this study was 1.4 msec. This might cause the steady-state activation curve to shift negatively more than 20 ~ 30 mV (Santoro et al. 2000). Second, previous study showed that the application of extracellular Ba$^{2+}$ (3 mM) decreased the amplitude of $I_h$ in human embryonic kidney cells although the half-activation potential of steady-state activation was not affected (van Welie et al. 2005). It is possible that the extracellular BaCl$_2$ (2 mM) has the same effect on decreasing the amplitudes of $I_h$ tail currents during the voltage-clamp recordings in CWCs (Fig. 3-7 A-B). When voltage levels of preceding test steps were close to the post step of -70 mV, tail currents elicited by post step would be too small to be distinguished from background noise. Thus, the steady-state activation would be shifted negatively by ignoring these activating tail currents.

Both experimental and simulation results showed that $I_h$ regulated the subthreshold responses of CWCs by contributing to the repolarization of $V_m$ following a SDP (Fig. 3-5, Fig. 4-13). CWCs show little intrinsic spontaneous activity in the presence of excitatory neurotransmitter antagonists (Waller and Godfrey 1994); therefore $I_h$ does not generate pacemaker activity in CWCs as in other neurons (Robinson and Siegelbaum
2003). However, similar repolarizing effects are found when $I_h$ contributes to the repolarization of $V_m$ following complex spikes in thalamic relay neurons (McCormick and Huguenard 1992b) and plays a role in regulating the repolarization of subthreshold responses produced by excitatory synaptic input (Magee 1999).

Despite the subthreshold regulatory role of $I_h$, no significant effect of $I_h$ on suprathreshold responses was observed either in experiments or in the model CWC (Fig. 4-13). However, when resting membrane potentials were hyperpolarized by the application of 20 μM ZD7288, elevating the resting potential levels by injecting constant inward currents showed that not only was the subthreshold response prolonged, with a slow return to resting level after the offset of stimulus, but also the suprathreshold response was converted from a simple spike followed by an ADP to a complex spike with AP spikes long after the offset of the depolarizing stimulus (Fig. 5-1). This phenomenon may result from the combined effects of two sources: one might be the facilitation of spiking by depolarizing membrane potentials (Fig. 3-13); the other might come from the offset of $K^+$ currents by blocking $I_h$, which, then, contributes to the repolarization of suprathreshold responses in CWCs. Unfortunately, this phenomenon is not sustainable under normal experimental conditions. The membrane potentials after spontaneous APs usually do not return to the resting levels, inducing sustained depolarization and finally killing cells. Thus, $I_h$ may contribute to the repolarization of suprathreshold responses in CWCs; but other experimental methods will be needed to maintain the electrochemical balances of recorded neurons.
**Figure 5-1:** $I_h$ affects both subthreshold and suprathreshold responses in CWCs when $V_m$ was depolarized. Upper: Responses to subthreshold stimuli under control conditions and in the presence of 20 µM ZD7288. After application of ZD7288, the resting potential was held at the same level as that under control conditions; and SDP was prolonged and exhibited a slow return to resting membrane potentials following the offset of the depolarizing stimulus. It confirmed that $I_h$ contributes to the repolarization of SDPs in CWCs. Lower: Responses to suprathreshold stimuli under control conditions and in the presence of 20 µM ZD7288. After application of ZD7288, the resting potential was held at the same level as that under control conditions; and a small increase in the amplitude of depolarizing current stimulus triggered a complex spike 20 msec after the offset of the stimulus. The repolarization after the first AP was so slow returning to the resting level that a second AP was elicited spontaneously even without stimuli. Dashed lines of $V_m$ represent for -70 mV.
5.3. Suprathreshold responses in CWCs

One striking difference between CWCs in this study and in previous studies (Ding et al. 1999; Golding and Oertel 1996, 1997; Manis et al. 1994; Zhang and Oertel 1993) is the inability of many CWCs to respond with complex spikes to suprathreshold stimuli (Molitor and Manis 2003). Differences between these studies include different rodent species, the use of whole cell or sharp-electrode recordings, and the ages of the animals used. Bursting units were observed in extracellular recordings from rat DCN (Waller and Godfrey 1994; Waller et al. 1996a), and complex spiking CWCs were observed during whole-cell recordings from mouse DCN (Tzounopoulos et al. 2004), suggesting that the lack of complex spiking CWCs cannot be attributed to species differences or recording methods. The electrophysiologic responses of DCN PCs obtained from P11 – P17 rats were identical to responses obtained from PCs in adult animals(Kanold and Manis 1999), and no significant differences in the electrophysiologic responses of CWCs were observed across the age range utilized for this study (P11 – P16). However, developmental changes in Ca\(^{2+}\) channels have been observed in rat cerebellar granule cells up to P21 (D'Angelo et al. 1997), and we cannot rule out the possibility that the electrophysiologic responses of CWCs are subject to maturational processes that extend beyond the age range used in this study.
Table 1: Comparison of Na\(^+\) mediated APs obtained from CWCs in adult (> 1 month old) guinea pig and in P11 – P16 rat DCN.

<table>
<thead>
<tr>
<th>Species</th>
<th>Spike height (mV)</th>
<th>Half width (ms)</th>
<th>Rise dV/dt (mV/ms)</th>
<th>Fall dV/dt (mV/ms)</th>
<th>Rise / fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig N = 20</td>
<td>64.6 ± 2.6</td>
<td>1.01 ± 0.11</td>
<td>230 ± 14</td>
<td>94 ± 6</td>
<td>2.59 ± 0.14</td>
</tr>
<tr>
<td>P11 – P16 rat N = 80</td>
<td>60.5 ± 1.4</td>
<td>0.77 ± 0.05</td>
<td>233 ± 8</td>
<td>105 ± 3</td>
<td>2.23 ± 0.05</td>
</tr>
</tbody>
</table>

Average AP measures (mean ± S.E.M.) obtained from the first AP of complex spikes during sharp electrode recordings from adult (> 1 month old) guinea pig brainstem slices (reproduced from table 1 of Manis et al., 1994) compared to those obtained from simple spikes or the first AP of complex spikes during whole cell recordings from P11 – P16 rat brainstem slices. Spike heights are measured from resting or baseline value; dV/dt values are maximal slopes obtained from the rising phase before and falling phases after the AP peak.
Regardless of developmental changes that may be responsible for the inability of some CWCs to produce complex spikes, the fast Na\(^+\) currents and Ca\(^{2+}\) currents that underlie APs and ADPs in the present study are likely to share many similarities with the fast Na\(^+\) and Ca\(^{2+}\) currents that underlie the complex spikes of CWCs observed during previous studies. Simple spikes observed in guinea pig CWCs were followed by ADPs (Manis et al. 1994), which are similar to the Ca\(^{2+}\)-dependent ADPs observed in the present study. Although Na\(^+\)-mediated SDPs have not been previously described, quantitative measurements of Na\(^+\)-mediated APs in P11 – P16 rat and adult guinea pig CWCs have an identical rising slope (Table 1), which is determined by fast Na\(^+\) current activation.

In contrast, APs in the present study have larger falling slopes and shorter half widths, suggesting an increased activation of K\(^+\) channels during the repolarizing phase. This hypothesis is supported by the observation that small amounts of 4-AP (50 – 200 µM) applied to simple spiking CWCs in P11 – P15 rats evoked complex spikes that strongly resemble those observed during recordings from adult guinea pig DCN (Fig. 3-11, 3-13); and simulation results demonstrated that changing the maximal conductance levels of voltage-gated K\(^+\) channels could affect the suprathreshold responses in a model CWC (Fig. 4-12). In the cell bodies of rat anteroventral cochlear nucleus (AVCN) neurons, densities of K\(_{v}1.1\) and K\(_{v}1.2\) voltage-gated K\(^+\) channels decrease after P21(Caminos et al. 2005). Thus, the inability of some CWCs to produce complex spikes may be due to the developmental regulation of suprathreshold response patterns by K\(^+\) channels, rather than maturational changes in the properties of the Na\(^+\) and Ca\(^{2+}\) channels described in the present study.
Experimental results also showed that a pre-step depolarization facilitated the generation of complex spikes in CWCs (Fig. 3-13), and additional studies in our lab have demonstrated that more complex spiking is observed when CWCs are held at more depolarized resting membrane potentials (unpublished results). Simulation results also show that when \( I_{\text{NaP}} \) was removed from the model, converting complex spikes into simple spikes, increasing the duration of injected current stimuli from 1 msec to 3 msec could recover the complex spikes by activating high-threshold Ca\(^{2+}\) currents. These findings suggested that experimental protocols used in this study may have substantial impact on suprathreshold responses in CWCs; reducing the portion of complex-spiking neurons by holding membrane potentials at levels greatly hyperpolarized relative to resting potentials or eliciting suprathreshold responses with brief (1 msec) stimuli.

5.4. Functional roles of complex spiking in DCN

The DCN possesses a cerebellar-like circuit that integrates both auditory and non-auditory inputs and projects multisensory information to higher auditory centers in the brain. Various pathways activate parallel fibers, the axons of granule cells that form excitatory synapses with the apical dendrites of PCs, and also provide excitatory synaptic input to CWCs that provide inhibition to PCs. \textit{In vitro} recordings from mouse DCN have demonstrated that complex spikes from CWCs elicit IPSP bursts in PCs, whereas simple spikes elicit a single IPSP (Golding and Oertel 1996). Simulated PC responses demonstrate that IPSP bursts are sufficient to deinactivate a fast transient K\(^+\) conductance and can substantially increase the first spike latency or first interspike interval produced by depolarizing stimuli, this can occur long after the postsynaptic membrane potential has
returned to resting levels (Fig. 1-3). *In vitro* recordings in rat DCN confirm the effects of inhibition upon spike timing in PCs (Kanold and Manis 2005), suggesting that the various suprathreshold responses of CWCs can have a profound impact upon the DCN output that is transmitted to higher auditory centers.

Recent studies on spike-timing dependent plasticity (STDP) demonstrate that long-term depression (LTD) could be induced at CWC/PF synapses by pairing excitatory postsynaptic potentials (EPSPs) and postsynaptic APs; the LTD at this synapse can be suppressed by blocking the postsynaptic entry of Ca\(^{2+}\) (Tzounopoulos et al. 2004). STDP in other neuronal systems also requires a postsynaptic elevation of [Ca\(^{2+}\)]\(_i\) (Bi and Poo 1998; Han et al. 2000; Magee and Johnston 1997; Tao et al. 2001). Although both simple and complex spikes are sufficient to induce LTD when paired with PF-mediated EPSPs, the induction of LTD could reduce complex spiking in response to subsequent PF-mediated inputs by reducing EPSP magnitude and duration. Furthermore, it is not known whether STDP will produce changes to intrinsic conductances such as I\(_{h}\) or I\(_{\text{NaP}}\), which could also have a profound effect on the suprathreshold responses of CWCs. Although the mechanisms that underlie synaptic plasticity in CWCs have not been thoroughly investigated, it is likely that various suprathreshold responses of CWCs and the resulting inhibition of PCs will play a significant role in it.
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