UNIVERSITY OF CALGARY

Synaptic Regulation of Intrinsic Dynamics in ELL Pyramidal Cells

by

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Abstract

A near ubiquitous feature of neuronal architecture is an extensive dendritic tree, an anatomical specialization that allows large numbers of synaptic inputs from presynaptic cells to influence the firing of a postsynaptic neuron. However, the dendrite is also able to directly influence somatic firing dynamics. Because of the electrotonic coupling between somatic and dendritic compartments, somatic events can charge dendritic membrane or trigger active dendritic spikes, promoting current flow which reflects back to the soma. This thesis is focused on dendrosomatic feedback, its regulation by inhibition, and the resulting effects on sensory processing in pyramidal cells of the electrosensory lobe (ELL) of the weakly electric fish *Apteronotus leptorhynchus*. I show that feedback arising from dendritic spike backpropagation has a multiplicative effect on the response gain of pyramidal cells. Moreover, gain can be regulated in a divisive manner by dendritic inhibition from GABA$_A$ receptor activation which decreases the amplitude of the dendritic spike. In contrast, GABA$_B$ receptor activation alters the timing of the dendritic feedback to cause a qualitative change in firing dynamics and a paradoxical increase in burst output. Thus, distinct subtypes of synaptic inhibition can have different nonlinear effects on the firing of the cell. I further model a direct feedback pathway known to recruit GABA$_B$ inhibition of pyramidal cells, whereas regulation of burst dynamics modulates frequency tuning and spike patterning, even when embedded in a dynamic network. Finally, I examine the intrinsic properties of pyramidal cell spike and burst discharge in relation to frequency tuning across three sensory maps of the ELL with known behavioural contributions. These interactions are
important in determining how this animal and central neural circuit encodes electric signals that are used to detect prey, examine its environment, and for social interactions. Since these interactions are often distinguishable by their frequency content, frequency tuning is of distinct ethological relevance in this system. I show that spike bursts that arise through dendritic feedback are correlated with low frequency tuning across the maps, and thus may contribute to specialized detection of prey-like events.
Epigraph

An education isn't how much you have committed to memory, or even how much you know. It's being able to differentiate between what you know and what you don't.

-Anatole France
Acknowledgements

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Dedication

I dedicate this thesis to my parents, whose remarkable patience and support have helped me throughout my life.
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<tr>
<td>ACSF</td>
<td>Artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AHP</td>
<td>afterhyperpolarization</td>
</tr>
<tr>
<td>AM</td>
<td>Amplitude Modulation</td>
</tr>
<tr>
<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>AP-5</td>
<td>D(-)-2-Amino-5-phosphonopentanoic acid</td>
</tr>
<tr>
<td>A.U.</td>
<td>Arbitrary Units</td>
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<tr>
<td>bAHP</td>
<td>Burst Afterhyperpolarization</td>
</tr>
<tr>
<td>C</td>
<td>Capacitance</td>
</tr>
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<td>Ca^{2+}</td>
<td>Calcium</td>
</tr>
<tr>
<td>Cl^{-}</td>
<td>Chloride</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CMS</td>
<td>Centromedial Segment</td>
</tr>
<tr>
<td>CLS</td>
<td>Centrolateral Segment</td>
</tr>
<tr>
<td>DAP</td>
<td>Depolarizing afterpotential</td>
</tr>
<tr>
<td>DAQ</td>
<td>Data Acquisition</td>
</tr>
<tr>
<td>DC</td>
<td>Direct Current</td>
</tr>
<tr>
<td>DFL</td>
<td>Deep Fibre Layer</td>
</tr>
<tr>
<td>DML</td>
<td>Dorsal Molecular Layer</td>
</tr>
<tr>
<td>DNL</td>
<td>Deep Neuropil Layer</td>
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<tr>
<td>DNQX</td>
<td>Dinitroquinoxaline-2,3-dione</td>
</tr>
<tr>
<td>EGp</td>
<td>Eminentia granularis pos posteriori</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>ELL</td>
<td>Electrosensory lateral line lobe</td>
</tr>
<tr>
<td>EOD</td>
<td>Electric Organ Discharge</td>
</tr>
<tr>
<td>FI</td>
<td>Frequency-current curve.</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GCL</td>
<td>Granule Cell Layer</td>
</tr>
<tr>
<td>GC2</td>
<td>Type II Granule Cell</td>
</tr>
<tr>
<td>GWN</td>
<td>Gaussian White Noise</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>IPSP</td>
<td>Inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>ISI</td>
<td>Inter-spike Interval</td>
</tr>
<tr>
<td>K⁺</td>
<td>Potassium</td>
</tr>
<tr>
<td>KAc</td>
<td>Potassium Acetate</td>
</tr>
<tr>
<td>LIF</td>
<td>Leaky Integrate and Fire</td>
</tr>
<tr>
<td>LS</td>
<td>Lateral segment</td>
</tr>
<tr>
<td>MS</td>
<td>Medial Segment</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>nP</td>
<td>Nucleus praeminentalis</td>
</tr>
<tr>
<td>PB</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>OI</td>
<td>Oscillation Index</td>
</tr>
<tr>
<td>OU</td>
<td>Ornstein-Uhlenbeck</td>
</tr>
<tr>
<td>PCL</td>
<td>Pyramidal Cell Layer</td>
</tr>
<tr>
<td>PL</td>
<td>Plexiform Layer</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PSI</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>RAM</td>
<td>Random amplitude modulations</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>S</td>
<td>Conductance (Siemens)</td>
</tr>
<tr>
<td>SK</td>
<td>Small Conductance Potassium Channel</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SNFP</td>
<td>Saddle node of fixed points</td>
</tr>
<tr>
<td>SNLC</td>
<td>Saddle node of limit cycles</td>
</tr>
<tr>
<td>STD</td>
<td>Short term depression</td>
</tr>
<tr>
<td>STA</td>
<td>Spike Triggered Average</td>
</tr>
<tr>
<td>StF</td>
<td>Stratus tractum fibrosum</td>
</tr>
<tr>
<td>TS</td>
<td>Torus Semicircularis</td>
</tr>
<tr>
<td>TTX</td>
<td>Tetrodotoxin</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>VML</td>
<td>Ventral Molecular Layer,</td>
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</table>
Chapter One: Introduction

1.1 General Introduction

Two families of fish (mormyrids and gymnotids) are known to have independently developed active electrosensory systems (Bullock and Heiligenberg 1986). Active electrosensory systems are distinguished from passive electrosensory systems by the use of a specialized electric organ (Arnold and Forest 1999). This organ generates electrical signals that are modified by the surrounding environment and picked up by receptors in the skin. Passive electoreception, such as that observed in skates, rays, and sharks rely on Ampullae of Lorenzi, detects weak electrical signals given off by living organisms. In comparison, active electrosensory systems rely on the generation of an electric organ discharge (EOD) to generate either a continuous wave or single pulses. Distortions of these carrier signals (e.g. Amplitude Modulations, AM) can be used to detect the presence and movement of objects, to distinguish living (conducting) objects from non-living (resistive) objects, and to detect the size and distance of such objects (von der Emde 1999). Because of the complexity and interconnectivity of mammalian sensory systems, detailed analyses of sensory processing at the single neuron level have many confounding variables which can make the interpretation of results difficult. The relative simplicity of sensory processing, of the signals themselves, and of the underlying neuronal architecture in the electric fish underlies its usefulness as an experimental model for research into the neural processing of sensory information. Neurons can be given precise, controlled sensory information in vivo with minimal interference from extraneous variables. Further, pyramidal cells that are the sole output of the medullary
electrosensory lateral line lobe (ELL) are only one degree removed from the sensory stimulus itself, and are capable of closely following the external AMs. This allows for the introduction of \textit{in vivo}-like stimuli into an \textit{in vitro} ELL slice preparation, easier access to cells, as well as more complex experiments in a reduced preparation, without sacrificing the physiological relevance of the experiment.

Both the gymnotid and mormyrid families of fish have been extensively studied since they were first described (Lissman 1958), but much of the study of the sensory system has focused on Gymnotiform fish, particularly \textit{Eigenmannia virens}\textsc{cens} (Heiligenberg 1991a) and \textit{Apteronotus leptorhynchus} (Berman and Maler 1999; Maler et al. 1991). Studies from the evolutionarily distant but functionally similar Mormyrid species have also made vital contributions to the understanding of sensory processing in this system (Bell 1982).

\subsection*{1.2 Circuitry and Anatomy}

The ELL is similar to the lateral line lobe of non-electric fish. Both systems take information from a modified waveform signal and encode it in a spike train. The ELL is a highly laminar structure (Figure 1-1), with four functionally and physiologically distinct maps- the Medial Segment (MS) does not encode active electrosensory signals, but instead receives inputs from passive electroreceptors (Zakon 1986). The remaining three segments participate in active electroreception and are distinguished by their optimal frequencies of processing- the centromedial segment (CMS) responds optimally to low stimulus frequencies ($< 5$ Hz), the centrolateral segment (CLS) is tuned to AMs of medium frequency (16-32 Hz). Finally, the lateral segment (LS) responds preferentially
**Figure 1-1 Regions of the ELL.**

Cresyl violet stained section of the hindbrain of *A. leptorhynchus*, with nuclear structures identified on a mirror image schematic diagram (taken from the gymnotiform fish atlas of Maler (1991)). The ELL is positioned between the brainstem and the EGp, the electrosensory division of the caudal lobe (LC) of cerebellum. Dashed lines in the ELL delineate the separate maps for electrosensory processing: the Medial (MS), Centromedial (CMS), Centrolateral (CLS) and Lateral (LS) segments. Abbreviations: Eminentia granularis pos posteriori (EGp), Dorsal molecular layer (DML), Ventral molecular layer (VML), Pyramidal Cell Layer (PCL), and Granule Cell Layer (GCL). Scale bar in mm. Adapted from Maler (1979).
to higher frequencies (> 32 Hz), although the maximal range is unknown (Shumway 1989a). Each segment is composed of multiple layers that contain morphologically and functionally distinct cells and feedback pathways (Maler 1979). I begin by discussing ELL pyramidal cells, the neurons which code amplitude modulations (AMs) generated by perturbations of the EOD, and provide a major portion of the output of the ELL.

1.3 ELL Pyramidal Cells

There are two major subtypes of pyramidal cells located in a distinct pyramidal cell layer, as determined by their morphology and in vivo firing characteristics. The morphological distinction is the presence or absence of a basilar dendrite. ELL pyramidal cells are exclusively innervated by P-units, which fire with some probability in each EOD cycle. Basilar pyramidal cells (BP, also called E-cells), receive excitatory input from the P-unit afferents and fire in response to upstrokes of the EOD AMs. In contrast, non-basilar pyramidal cells (NBP, or I-cells), receive indirect inputs from P-unit afferents through granule cell inhibitory interneurons, the GC2. This provides a mechanism whereby I-cells are inhibited by AMs of the fish’s EOD while downstrokes in EOD AM result in depolarization of I-cell membrane voltage (Figure 1-2). This allows the animal to encode both upward and downward transients in the external EOD AMs through separate neuronal subtypes. The ELL however, also contains a myriad of local interneurons which I discuss next.

The pyramidal cells and interneurons of the ELL, as well as the two most important sources of descending feedback are described in Figure 1-3. The structure of
Figure 1-2  E- and I-type ELL pyramidal cells are able to encode up or downstrokes of an electro sensory stimulus.

Electrosensory information carried by EOD AMs (red) is encoded by P-unit activity, and projects excitatory input to either E-type pyramidal cells or GC2 granule cells. Direct input to E-cell basilar dendrites allows upstrokes in the EOD AM (red trace) to depolarize E-cell membrane voltage. GC2 granule cells allow an inverted version of the EOD AM to be passed to I-type pyramidal cells, such that downstrokes in the EOD AM cause upstrokes in the pyramidal cell membrane voltage (black trace), thereby driving spikes.
Figure 1-3 General circuitry of the ELL.

P-unit afferents synapse on ELL E-type pyramidal cells, or onto GC2 granule cells which then contact I-type pyramidal cells. Pyramidal cell axons give rise to the Lateral Lemiscus (LL) in projecting to the contralateral nucleus Praeminentialis (nP), which contains three cell types. nP stellate cells project directly back to the ELL via the Stratus tractum fibrosum (StF), sending glutamatergic synapses to pyramidal cells and to VML cells. Bipolar cells also project via the StF, giving rise to GABA_B synapses on pyramidal cell dendrites. Multipolar cells project to the Eminentia granularis pos posteriori (EGp), where granule cells give rise to the descending parallel fibres. The parallel fibres send glutamatergic synapses to apical dendrites of pyramidal cells and VML cells, as well as onto ELL stellate cells. VML cells project GABAergic (GABA_A) synapses to the proximal apical dendrite of pyramidal cells, while GC2 granule cells inhibit the soma of pyramidal cells. Excitatory synaptic connections are shown in red, inhibitory synapses are shown in blue.

the ELL, from ventral to dorsal, consists first of the deep fibre layer (DFL), where the primary afferents are located. The next most ventral layer is the granule cell layer (GCL) which contains granule cells, spherical cells, ovoid cells (not shown), and the basilar dendrites of “E-cells”, as well as the somata of a class of pyramidal cells referred to as deep basilars (Bastian and Courtright 1991; Bastian and Nguyenkim 2001). The plexiform layer (PL) is formed by the axons of pyramidal cells projecting to the midbrain and gives rise to the major output of the ELL, the lateral lemniscus (LL). The pyramidal cells send apical dendrites dorsally through the stratus tractum fibrosum (StF), a dense fibre bundle originating from the contralateral midbrain. The apical dendrites extend dorsally into the ventral molecular layer (VML) and then the dorsal molecular layer (DML). The primary neuronal subtype in the VML are the VML cells, a class of inhibitory interneuron discussed in more detail in Chapter 3. The VML also contains the dendrites of pyramidal cells positioned more ventrally in the pyramidal cell layer and granule cells. The DML contains parallel fibres from the ipsilateral and contralateral cerebellum, as well as inhibitory stellate cells. The more dorsal parallel fibres project from the contralateral eminentia granularis pos posteriori (EGp), while the more ventral fibres project from the ipsilateral EGp (Sas and Maler 1987).

1.4 Feedback circuits

The ELL pyramidal cells receive three major sources of input. Feedforward input to pyramidal cells comes from the primary electro sensory afferents (P-units) that trifurcate upon entry into the ELL and feed directly to the basilar dendrites of E-cells in
each of the three active electrosensory segments (Lannoo et al. 1989). P-unit axons also synapse onto two types of granule cells, type 1 (GC1, not shown) and type 2 (GC2). The sole output of the ELL is through the LL, projecting rostrally to the contralateral nucleus praeeminentialis (nP) (Maler 1979; Maler et al. 1982) and to the Torus Semicircularis (TS). The nP contains stellate, multipolar, and bipolar cells (Sas and Maler 1983). The nP stellate and bipolar cells project directly back to the contralateral ELL via the StF (Berman and Maler 1999; Plant 1994) and make up a direct and topographic feedback pathway. A further indirect pathway is mediated by nP multipolar cells, which project mossy fibers along the lateral border of the overlying electrosensory lobe of cerebellum, the Eminentia Granularis posterior (EGp), to drive granule cells that give rise to the parallel fibre feedback projecting to the ELL molecular layers.

1.4.1 Direct feedback

The StF pathway couples to the ELL with both direct reciprocal excitation (via nP stellate cells) and inhibition (via nP bipolar cells): firing of pyramidal cells excites cells in the nP which then feed back to the ELL (Maler et al. 1982). The bipolar cells project in a diffuse fashion (Maler and Mugnaini 1994) to many pyramidal cells where they release gamma-aminobutyric acid (GABA), and activate both a GABA<sub>B</sub> receptor mediated conductance and a GABA<sub>A</sub> receptor mediated component (Berman and Maler 1998c). Although under spontaneous (e.g. unstimulated) conditions the bipolar cells fire irregularly and in an uncoordinated fashion, under certain communication-like stimuli the cells fire in slow oscillations, appearing as a 30 Hz peak in the power spectrum of the pyramidal cell spike train (Doiron et al. 2003a). Stellate cells of the nP project
topographically back to the pyramidal cells and are glutamatergic (Sas and Maler 1983; 1987), with a strong N-methyl-D-aspartate (NMDA) receptor mediated component (Maler and Monaghan 1991) as well as a fast alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated and occasionally gap junction mediated components (Berman and Maler 1999). Gap junctions have only been reported in non-basilar pyramidal cells, however (Maler et al. 1981), and the computational implications of this are unknown. The StF projections also synapse onto VML cells, although the time course and pharmacology of the conductance is unknown.

Importantly, VML cell axons project to the proximal apical dendrites of pyramidal cells within 50 μm of their soma, and form an “inverse cone” shape as they progress through the StF, reaching the soma in 5% of observed cases (Maler et al. 1981). This forms a local microcircuit, where each VML cell is associated with a pair of pyramidal cells, one basilar (E-cell) and one non-basilar (I-cell).

Although *in vitro* examinations of the StF feedback show this pathway can provide both excitatory and inhibitory feedback (Berman and Maler 1998c; Lewis and Maler 2002; 2004), *in vivo* examinations show that the net effect of the StF feedback systems is inhibitory, controlling response gain to electrosensory input. Thus, after lesioning of the StF, the pyramidal cell response to external stimulation increases drastically (Bastian 1986a; b).

1.4.2 *Indirect feedback*

The nP multipolar cells project to the EGp where they innervate cerebellar granule cells. In comparison to the closed loop feedback systems described above, the
granule cells also take in input from higher centres as well as from other senses. The EGp receives minor bilateral tracts projecting from the dorsomedial optic tract, dorsal tegmental nucleus, lateral tegmental nucleus, all three clusters of the paramedian reticular nucleus, the octaval and octavolateralis nuclei, the perihypoglossal nucleus, the lateral reticular neurons, the medullary reticular formation, and the funicular nucleus.

Serotonergic cells of the nucleus raphe dorsalis have significant bilateral projections to the EGp. Cells in the contralateral inferior olive project strongly to the EGp. Finally, there is a minor contralateral projection from the pretectal nucleus (Sas and Maler 1987). Thus, although the EGp receives a major component of input from the ELL, this is far from the sole innervation of the cells that give rise to the parallel fibre feedback pathway.

EGp granule cells give rise to the descending parallel fibre projection in the VML and DML. These parallel fibres are functionally and anatomically similar to cerebellar parallel fibres, and form glutamatergic synapses onto dendrites throughout the three maps. Fibres running in the most distal regions are from the contralateral EGp, while those coursing more ventrally in the VML project from the ipsilateral side (Sas and Maler 1987). Parallel fibres synapse on the apical dendrites of pyramidal cells as well as those of VML stellate cells, which in turn give rise to fast GABA_A inhibitory synapses on the pyramidal cell dendrites. Parallel fibres also have glutamatergic synapses onto the dendrites of GC2, polymorphic, and VML cells cells (not shown) (Wang and Maler 1994).
1.5 Bursting in the ELL

Pyramidal cells of the ELL can fire in stereotyped bursts of spikes (Turner et al. 1994). This bursting relies on active sodium spike backpropagation along dendrites (Lemon and Turner 2000). Immunofluorescent staining has shown the active regions of dendrites to be localized in patches, with intervening regions of relatively passive membrane (Turner et al. 1994). The apical dendrites seem to be the sole contributors to the dendrosomatic feedback which underlies bursting (Lemon and Turner 2000), and models replicating the bursting behaviour are able to function based solely on the contribution of apical dendrites (Doiron et al. 2002; Doiron et al. 2001a; Fernandez et al. 2005b). The bursting works by a “ping-pong” mechanism in which a somatically initiated spike propagates along the apical dendrite, activating sodium channels that in turn cause current flow back to the soma (Figure 1-4A). This creates a depolarizing after potential (DAP) in the soma that increases the net excitation. This leads to the firing of another spike, with a shorter interspike interval (ISI), and a broadening of the dendritic spike that develops in a frequency-dependent manner (Figure 1-4B). This process continues until the somatic ISI falls within the refractory period of the dendritic sodium channels (Fernandez et al. 2005b; Lemon and Turner 2000; Noonan et al. 2003) resulting in an abrupt failure of dendritic backpropagation. This dendritic spike broadening, initially thought to be caused primarily by the inactivation of K\(^+\) currents (Doiron et al. 2002; Doiron et al. 2001b; Rashid et al. 2001b), was recently shown to be driven by the cumulative inactivation of dendritic Na\(^+\) currents (Fernandez et al. 2005b).

Pyramidal cells shift from a tonically firing to a bursting mode with applied current, and the bursting response is also influenced by the strength of the input current.
Figure 1-4 Conditional backpropagation and bursting in the ELL.

A) Schematic diagram of conditional backpropagation underlying burst discharge in ELL pyramidal cells. A somatic spike propagates into the apical dendrite, where it activates dendritic sodium channels. The resulting inward dendritic current conducts back to the soma, generating a depolarizing afterpotential (DAP). B) Over the course of the burst, the DAP grows in a frequency-dependent manner, contributing to cell excitability and decreasing the interspike interval (ISI). Eventually the ISI falls within the dendritic refractory period and the spike fails to backpropagate, terminating the burst when the depolarizing influence of the dendritic spike is lost. Figure from Lemon and Turner (2000).
driving it. A non-linear dynamical analysis of bursting showed that after the loss of the stable fixed point (through a saddle node of fixed points, SNFP), a stable oscillation resulted. With increased current injection there is a second bifurcation, a saddle node bifurcation of limit cycles (SNLC), which leads to an unstable limit cycle at higher levels of current injection (Doiron et al. 2002; Fernandez et al. 2005b). This sensitivity to small variations in input currents underlies the range of responses possible in ELL pyramidal cells (Laing et al. 2003), allowing bursting that serves both to indicate feature detection within the stimulus, as well as to provide an estimate of the stimulus itself (Doiron et al. 2007; Gabbiani and Metzner 1999; Gabbiani et al. 1996; Krahe and Gabbiani 2004; Oswald et al. 2007).

1.5.1 Bursting is conserved across multiple species of Gymnotiform fish

Studies on the biophysics and dynamics of burst firing in the ELL have been performed primarily in *Apterontotus leptorhynchus* (Doiron et al. 2002; Fernandez et al. 2005b; Lemon and Turner 2000; Noonan et al. 2003; Turner et al. 1994). In order to examine whether similar bursting can be observed across multiple species of fish, I performed *in vitro* slice recordings from two other species of gymnotid fish (*Apterontotus albifrons* and *Eigenmannia virescens*). Figure 1-5A shows the consensus phylogeny for the order Gymnotiform, taken from (Alves-Gomes 1999). A representative example of burst firing in *A. leptorhynchus* is shown in Figure 1-5B. As described above, repetitive firing of bursts is observed above a threshold level of current injection. A similar phenotype of burst discharge can be observed in ELL pyramidal cells of *A. albifrons* (Figure 1-5C). As these two species are closely related (Figure 1-5A), we also performed
**Figure 1-5 Bursting is conserved across many Gymnotiform species.**

A) The consensus phylogeny for order Gymnotiform, taken from (Alves-Gomes 1999).

B) Representative example of burst firing in ELL pyramidal cells of *Apteronotus leptorhynchus* in *vitro*. Repetitive firing of bursts is observed after sufficient current injection (0.45 nA) promotes a conditional backpropagation of dendritic spikes. C) A similar phenotype of burst discharge can be observed in pyramidal cells in an *in vitro* slice preparation of the closely related species *Apteronotus albifrons* (0.7 nA current injection). D, Representative example of bursting in an ELL pyramidal cell of the more distantly related Gymnotiform, *Eigenmannia virescens* (0.25 nA current injection) maintained *in vitro*. 
these experiments on a more distantly related Gymnotiform, *E. virescens*. Burst discharge was also observed in *E. virescens*, showing that burst firing is likely conserved across many wave-type electric fish (Mehaffey et al. 2008a)(Figure 1-5D). Note that *E. virescens* have a myogenically derived electric organ, whereas Apterontids have a neurogenically derived electric organ. Thus, although they most likely developed active electrosensation independently, they both show a similar bursting phenotype in ELL pyramidal cells. Interestingly, a similar pattern of burst discharge can also be observed in *Gymnotus carapo* (Angel Caputi, personal communication), a pulse-type fish distantly related to *A. leptorhynchus*. Although conservation of the bursting phenotype does not necessarily prove conservation of the burst mechanism, it suggests that burst discharge is a common adaptation for electrosensory processing in ELL pyramidal cells, and is thus likely to contribute to effective coding of electrosensory inputs.

**1.5.2 Types of bursting observed in vitro and in vivo**

Two types of bursting have been described in ELL pyramidal cells. The type discussed above, where the bursting is observed in response to a square wave current injection is mediated by gradual shifts in the characteristics of the backpropagating action potential (Doiron et al. 2002; Fernandez et al. 2005b) and can be observed both in vitro and in vivo (Oswald et al. 2004; Turner et al. 1994). Under spontaneous (e.g. unstimulated) in vivo recording conditions, a subset of cells tend to fire in short bursts of activity (2-3 spikes) rather than single spikes (Bastian and Nguyenkim 2001; Gabbiani et al. 1996). This bursting was correlated with the size of the pyramidal cell dendritic arbour and required parallel fibre feedback, indicating a net excitation coming from the apical
dendrites (Bastian and Nguyenkim 2001). This study also showed a distribution of burst phenotypes depending on the location of pyramidal cells with the PCL (Bastian and Courtright 1991; Bastian and Nguyenkim 2001). Pyramidal cells located dorsally in the PCL (e.g. superficial pyramidal cells) have a large dendritic tree reaching to the distal VML and therefore receive feedback inputs from both the contralateral and ipsilateral EGp via the parallel fibres. These cells burst in the fashion described by Bastian and Nguyenkim (2001). Intermediate pyramidal cells reside in the middle of the PCL, and have a smaller dendritic arbour that does not reach the DML. This indicates that they receive primarily ipsilateral parallel fibre feedback. Finally, deep basilar pyramidal cells are located just dorsal to the deep neuropil layer, have small basilar dendrites, and have apical dendrites extending to, but rarely past the level of the StF. These cells receive the least amount of feedback, exhibit the least plasticity of synaptic transmission, and track the EOD the most faithfully, a phenomenon which has been suggested to be required for plasticity in other pyramidal cell classes (Bastian et al. 2004). With respect to output, deep basilar pyramidal cells burst strongly to input in vivo (M. Chacron, personal communication), and can be observed undergoing the full slow burst dynamic first described in vitro (Oswald et al. 2004). Recent work suggests that the two mechanisms are related, in that cells showing the highest propensity to burst with the full slow dynamic also fire more pairs of spikes in response to time-varying inputs in vitro (Mehaffey et al. 2008b).
1.5.3 Bursting in response to time-varying inputs

A recent line of investigation has been to examine the role of bursts in sensory coding. Bursts have been shown to be informative in many sensory systems, including the auditory system (Eggermont and Smith 1996) and the visual system (Lesica and Stanley 2004; Lesica et al. 2006). Further, the discharge of bursts is often related to frequency tuning (Butts et al. 2007; Eggermont and Smith 1996; Ellis et al. 2007b; Lesica and Stanley 2004; Lesica et al. 2006). When driven with time-varying inputs, such as filtered Gaussian noise (see Methods), the resulting spike train in ELL pyramidal cells can be partitioned into two clear populations (Doiron et al. 2007; Oswald et al. 2004; Oswald et al. 2007). The first is a population of fast ISIs due to dendritic feedback from the burst mechanism, and the second is a population of slower ISIs. The ISI histogram has a clear nadir separating these two populations of ISIs, which requires the dendritic feedback which underlies the burst mechanism (Oswald et al. 2004). Importantly, these two separate populations of ISIs show distinct frequency preferences. Bursts of spikes are preferentially driven by slow stimulus components, while isolated spikes fire in response to fast stimulus components (Doiron et al. 2007; Oswald et al. 2004; Oswald et al. 2007). Thus the frequency content of a continuous time-varying stimulus is partitioned into two populations of spikes. This frequency content is relevant to the electrosensory scene, as high frequency stimulus components tend to be associated with communication signals or with the presence of conspecifics (Middleton et al. 2006; Zupanc et al. 2006), while low frequency events tend to be associated with environmental signals (MacIver et al. 2001).
1.6 The ELL contains repeating but specialized topographical maps

As mentioned above, the region of the ELL processing AMs contains three repeating topographic maps. There are measurable differences in pyramidal cell discharge across the ELL maps *in vivo* and *in vitro*. These are attributable to both the underlying anatomy (Shumway 1989b) and the differential distribution of ion channels underlying spike and burst discharge (Deng et al. 2005; Ellis et al. 2007b; Fernandez et al. 2005b; Mehaffey et al. 2006). *In vivo* the three maps receiving P-unit afferent input fire preferentially to specific frequencies of input (Shumway 1989a). Other physiological differences between the three maps (CMS to LS) include variability in the size of receptive fields (small to large), strength of surround inhibition (strong to weak), sensitivity to differences in AM (low to high), rate of adaptation (slow to fast), latency to maximum response (long to short), the degree of convergence by P-unit inputs, and in the frequency preference observed *in vivo* (low pass, band pass, and high pass filters, CMS, CLS, and LS, respectively) (Shumway 1989a) (L. Maler, personal communication). Taken together, the data suggest that the cells vary both in their preferred frequencies and in the precision of information they deliver. The LS would be expected to provide a lower resolution description of high frequency events and to quickly adapt. In comparison, the CMS would be expected to show the highest resolution of low frequency events, with less adaptation and a higher spatial resolution. I-cells have been shown to match this pattern, although E-cells have been demonstrated to perform more poorly than I-cells at detecting up or downstrokes in EOD amplitude (Metzner et al. 1998). Further, there is no known direct connections between maps, and different maps have been suggested to mediate behaviourally separate functions (CMS provides jamming avoidance, LS is
specialized for communication signals) (Metzner and Juranek 1997). It has been suggested that the three maps may therefore function independently to mediate different behaviours.

The anatomical differences that correlate to these preferences in input include a decrease both in the number of pyramidal cells and of their associated inhibitory neurons (ovoid, polymorphic, granule and multipolar cells, Berman and Maler 1999; Shumway 1989b) proceeding laterally across the ELL. These GABAergic inputs contribute to spatial and temporal processing across maps in the ELL and allow for control by descending inhibitory feedback (Maler and Mugnaini 1994; Shumway 1989b). In contrast, the number of VML cells increase from the CMS to the LS, as does the amount of convergence by P-unit afferents, indicating that individual cells in the CLS and LS monitor electrosensory information from a larger area of the skin surface (Shumway 1989a; b), and are more likely to be regulated by the VML cells. The function of VML cells is currently unknown, although they have been suggested to have a role in temporal filtering by the descending parallel fibre feedback (Berman and Maler 1999), and they have been implicated in regulating the gain of the firing response of pyramidal cells (Mehaffey et al. 2005), Chapter 3).

It has been shown that activation of dendritic GABA receptors can dramatically reduce backpropagating action potentials in mitral cells and neocortical cells (Larkum et al. 1999; Lowe 2002; Xiong and Chen 2002). Previous studies have shown that direct negative current injection through a patch electrode on the apical dendrite can prevent backpropagation (and therefore bursting) in neocortex (Williams and Stuart 1999). In the ELL pyramidal cell, we have a distinct neuron whose anatomy, pharmacology, and
subcellular localization of excitatory and inhibitory synaptic inputs are well established. The spatially distributed mechanism of bursting in pyramidal cells provides a powerful mechanism by which dendritic GABAergic input could gate firing behavior in a nontrivial fashion. Further, the location of VML cell synapses along the dendrite rather than the soma suggests that it may regulate dendritic activity without excessively interfering with the site of spike initiation (Holt and Koch 1997). This would be a novel variation on the modulatory agents previously shown to gate bursting in corticopontine cells by norepinephrine and acetylcholine (Wang and McCormick 1993), which can shift neurons into a purely bursting mode. Such regulating of the underlying intrinsic driving currents could, however, drastically increase responsiveness of the cell to any inputs (and thereby cause false detections), an unwanted side effect in a sensory system. A modulation of the firing dynamics without a shift in rheobase would maintain or enhance sensitivity without sacrificing accuracy in feature detection.

1.7 Frequency and spatial content of natural signals

Low frequency events to which the fish is likely to be exposed to arise primarily from two major classes of events. The first is environmental signals, generated when the animal moves past objects in the electrosensory surrounding, which are generally low frequency (Maclever et al. 2001). The rate of movement is also varied according to the ability of the fish to detect nearby objects, which is in turn a factor of the sensing volume of the fish (Snyder et al. 2007). Another source of low frequency events occurs in a social context: the presence of a conspecific of the same gender will generate large amplitude beats (rhythmic AMs due to interference between the two signals) to avoid jamming of
electrosensory performance. Fish of a different gender emit EODs at widely different frequencies, creating lower amplitude, higher frequency beats as the difference in EODs is greater (often > 300 Hz). In fact, behaviorally these fish shift their EOD frequency in order to avoid low-frequency beats generated by same sex conspecifics. In the related species *Eigenmannia* the final, shifted beats are between 20-40 Hz (Heiligenberg 1973). Similar beat frequencies are reported between schools of Apteronotids in field recordings, suggesting that both species avoid low frequency beats (E. Fortune, personal communication). Thus, if the animal is adapted to the presence of a conspecific, the beat frequencies will therefore be high frequency, and only contain low frequency power transiently (> 10 Hz). Communication signals are brief high frequency events (Zupanc et al. 2006) that can transiently synchronize or desynchronize receptor afferent activities (Benda et al. 2006). Such brief transients will necessarily create depolarizations in ELL pyramidal cells with primarily high frequency content due to the brief duration. Thus both on-going beats and communication signals are likely to eventually generate high frequency events. In small groups of fish, the beats become more complex and the envelope of the beats is coded (Middleton et al. 2006), which again contains primarily high frequency content.

Another important factor distinguishing these two classes of sensory signals is their spatial extent- communication signals from other fish tend to be spatially uniform, as the electric field of a conspecific generates spatially extensive distortions along the surface of a neighbouring fish. In contrast, prey and electrosensory signals tend to be more spatially localized and uncorrelated with activity in nearby receptive fields. This leads to the summary presented in Figure 1-6, which diagrams these two factors (spatial
Figure 1-6 Diagram of the behavioral relevance of the frequency content and spatial distribution of electrical fields induced by different environmental signals.

Shown is a general classification of the frequency content of electrical signals arising from prey items (local) or interactions between conspecifics (global). Socials signals have significant spatial correlations, and are referred to as ‘global’ signals as they stimulate a large proportion of receptive fields with a similar input. Some social signals are low frequency, occurring between conspecifics of the same gender. These signals are generally transient, however, as the fish shift their EOD frequency to remove low frequency (<20 Hz) interference from beats, a process referred to as a Jamming Avoidance Response (JAR). Communication calls arising from perturbations of a fish’s EOD signal contain high frequency power, and have significant spatial correlations. In comparison, local signals, such as prey and environmental signals contain low frequency power. There are no known high frequency low signals. Fish drawings taken from Chacron et al. (2003).
extent and frequency content) in the context of their behavioural relevance. Spatially extensive signals can contain high frequency power (e.g. communication) or lower frequency power (e.g. jamming). When two conspecifics of the same gender meet, they may have similar EOD frequencies, creating large “beats” at the difference frequency (df) that interfere with electrolocation. This is generally transient, however, as the animals shift their EOD frequencies to obtain dfs > 10 Hz. The majority of communication signals in a group of fish are therefore > 10 Hz and spatially extensive. In contrast, low frequency events are usually spatially localized, and there are no known stimuli which would create large, high frequency local distortions of the EOD.

1.8 Gain Control

Control of a cell’s response gain (where gain is usually in Δ Hz/nA) is a major computational principle of sensory processing (Salinas and Thier 2000) that has been implicated in object invariance (Salinas and Abbott 1997) and coordinate transformations (Salinas and Abbott 2001). Inhibitory input alone fails to control gain, but rather induces a subtractive inhibition of the frequency/current (FI) curve by changing the rheobase for firing (Figure 1-7A) rather than the gain of the response (Holt and Koch 1997). Achieving gain control in these systems requires divisive inhibition. Divisive inhibition causes a change in the rate of response to an input, decreasing the slope of the neuron’s FI curve (Figure 1-7B). Numerous attempts have been made to examine the synaptic events required for divisive gain control. Although some theoretical studies had suggested that GABA_A mediated inhibition could achieve this (Rose 1977), later experimental and theoretical studies showed that somatic shunting inhibition has a purely
Figure 1-7 Gain control and sensory tuning curves.

A) Schematic diagrams to illustrate the difference between divisive and subtractive inhibition as it pertains to FI curves. Subtractive inhibition shifts the rheobase for spike discharge, while divisive inhibition changes the gain without shifting rheobase. B) Effects of divisive inhibition on a hypothetical tuning curve. Divisive inhibition (A) leads to a broadening of the tuning curve, extending the range of stimuli the cell can encode, indicated by dashed lines. a.u, arbitrary units.
subtractive effect, shifting the start of the FI curve, rather than modulating gain of the cell (Chance et al. 2002; Gabbiani et al. 1994; Holt and Koch 1997; Ulrich 2003). More recent studies have shown various parameters that can generate divisive effects. Somatic inhibition with noise can generate a weak divisive effect, but has a primarily subtractive effect in a modelled ELL pyramidal cell (Doiron et al. 2001a). A relatively balanced amount of somatic excitatory inputs combined with somatic inhibitory inputs was sufficient to generate divisive inhibition in neocortical pyramidal cells (Chance et al. 2002), as was a combination of dendritic excitation and somatic inhibition in a large compartmental model (Prescott and De Koninck 2003). The system seen in *A. leptorhynchus* is more similar to that described by Prescott (2003), based on both the anatomy and the physiology described above- EPSPs conduct forward through a dendrite, and interact with direct somatic inhibition to control the response gain (Bastian 1986a; b).

ELL pyramidal cells represent the first level of processing of electro-sensory information, receiving inputs from P-units which faithfully encode the EOD AMs (Chacron et al. 2001; Chacron et al. 2000; Nelson et al. 1997; Ratnam and Nelson 2000), which can accurately translate into perturbations of the pyramidal cell’s membrane voltage (Chacron et al. 2003; Middleton et al. 2006). Thus, the FI plot of pyramidal cells is a significant predictor of the expected response to an *in vivo* like stimulus. Moreover, modulation of the gain of the FI relationship can change the responsiveness of the cell without requiring modification of the input current resulting from stimulation of the receptive fields. As the degree of convergence of primary afferent input increases laterally across the maps, the receptive fields will widen and thus the overlap in their respective tuning curves will increase. Analysis of population codes for sensory
processing has shown that sharper tuning curves can be beneficial, although not in all situations (Pouget et al. 1999), and differences have been shown depending on the dimensionality of the stimulus being encoded (Zhang and Sejnowski 1999). Thus there are contexts in which either a wide, or a narrow, tuning curve can be maximally beneficial, and the ability to temporarily increase or decrease the gain of the tuning curve would be beneficial for some types of information processing. The ability to filter inputs and to control gain is a common observation throughout this and other nervous systems. The regulation of gain would be expected to allow for drastic switching in the tuning properties of the cell on a brief time scale. We examine these facets of gain control in the electric fish model system. It is hoped that the study of these processes in a well understood and well defined system will shed light on the possible mechanisms used for similar computation in higher processing centres.

1.9 Thesis Hypotheses

This thesis is focused on dendrosomatic feedback and the synaptic regulation of intrinsic firing dynamics. I examined these phenomena through in vitro slice experiments and numerical models in order to elucidate the dynamics of the interplay between dendrites and somata of pyramidal cells that govern burst discharge and gain control. The hypotheses tested can be summarized as follows:

1. Dendritic activity multiplicatively regulates the firing response of ELL pyramidal cells though dendrosomatic feedback (Chapter 3).
2. The influence of dendritic spikes can be decreased by GABA\textsubscript{A} mediated inhibition to divisively modulate the gain of pyramidal cell firing (Chapter 3).

3. Burst output and frequency selectivity is regulated through modulation of spike backpropagation by dendritic GABA\textsubscript{B} receptor activation (Chapter 4).

4. The nP-ELL direct feedback pathway regulates feature selection and frequency selectivity of pyramidal cells through GABA\textsubscript{B} mediated inhibition (Chapter 5).

5. Postsynaptic membrane properties effect differential burst output and frequency selectivity across the three tuberous maps of the ELL (Chapter 6).

The methodology described in Chapter 2 and the experiments described in Chapters 3 through 6 are designed to directly test these hypotheses to understand the relationship between dendrosomatic feedback, firing response phenotype, gain, and the frequency selectivity of individual pyramidal neurons.
Chapter Two: Methods

2.1 Electrophysiology

2.1.1 Preparation of slices

Weakly electric Brown Ghost knifefish (*Apteronotus leptorhynchus*), Black Ghost knifefish (*Apteronotus albifrons*), or glass knifefish (*Eigenmannia virescens*) were obtained from local importers and maintained at 26-28°C in fresh water aquaria in accordance with protocols approved by the University of Calgary Animal Care Committee. All experiments other than those described in Figures 1-2 were performed on *A. leptorhynchus*. All chemicals were obtained from SIGMA (St. Louis, MO) unless otherwise noted. In all cases recordings were obtained from separate pyramidal cell somata or apical dendrites in *in vitro* slices. Animals were anaesthetized in 0.05% phenoxy-ethanol, and ELL tissue slices of 250-350 μm thickness were prepared as previously described (Turner et al. 1994). Slices were maintained by constant perfusion of ACSF (~1-2 ml/minute) and superfusion of humidified 95% O₂, 5% CO₂ gas. ACSF contained (in mM): 124 NaCl, 3 KCl, 25 NaHCO₃, 1.0 CaCl₂, 1.5 MgSO₄ and 25 D-glucose, pH 7.4. Pharmacological agents were ejected locally from a pressure micropipette containing HEPES-buffered ACSF (in mM): 148 NaCl, 3.25 KCl, 1.5 CaCl₂, 1.5 MgCl₂, 10 HEPES, and 25 D-glucose, pH 7.4.

2.1.2 Stimulation and recording procedures

Glass microelectrodes were backfilled with 2 M KAc (pH 7.4; 90-120 MΩ
resistance) and in some recordings contained 2% Neurobiotin (Vector Labs). In general recordings were made from the two largest ELL segments receiving primary afferent input from P-units (CLS and CMS) and data were grouped (Maler et al. 1991), except for Chapter 6 where differences across maps were quantified. Recordings from specific maps were made through visual observation of the boundaries of each map, which are clearly delineated in the *in vitro* slice preparation. Somatic recordings were made from the pyramidal cell layer and dendritic recordings at the boundary of the stratum fibrosum (StF) and molecular layer containing the apical dendrites of pyramidal cells (Turner et al. 1994). When neurons had been filled with Neurobiotin, the slices were fixed in 4% paraformaldehyde in phosphate-buffered saline and subsequently reacted with streptavidin-conjugated Cy3 in 1.0% Triton-X 100 and 2% DMSO for 48 hrs for visualization on an Olympus FV300 BX50 confocal microscope. We were therefore able to classify pyramidal cells as basilar (E-cell) or nonbasilar (I-cell) types based on anatomy.

Recordings were digitized using a NI PCI-6030E DAQ board (National Instruments, Austin TX). Intracellular stimuli were delivered, and data was recorded using custom software based on the Matlab data acquisition toolbox (Mathworks, Natick MA). Dendritic and somatic recordings were distinguished by the placement of the electrode and from the spike waveform (dendritic spikes in the ELL display a minimal afterhyperpolarization (AHP) and a wider half-width than somatic spikes, Turner et al. 1994). Specific antidromic activation of pyramidal cells was accomplished by stimulating the plexiform layer (PL) using a bipolar tungsten electrode (Turner et al. 1994). For dendritic recordings, the electrode was placed in or slightly above the StF. We therefore believe our dendritic recordings to be within ~ 100 μM of the soma, although exact
distances cannot be precisely known for each recording. Stimulation of the nP inhibitory
inputs in the StF feedback pathway was accomplished by placing the stimulating electrode
at its ventral edge in the presence of 6,7-Dinitroquinoxaline-2,3-dione (DNQX), and D(-)-2-
Amino-5-phosphonopentanoic acid (AP-5) to block excitatory neurotransmission, as
previously reported (Berman and Maler 1998a).

Random amplitude modulations (RAMs) were evoked using intracellular current
injected as white noise low-pass filtered to 0-60 Hz. As sensory input in this frequency
range is well tracked by the membrane potential of pyramidal cells (Chacron et al. 2003;
Middleton et al. 2006), these intracellular current injections serve as good mimics of afferent
input associated with natural electrosensory signals. RAMs were given near threshold for
firing of the cell, and the standard deviation (SD) of the waveform was adjusted to give
firing rates of 10-30 Hz, which is typical of these cells in vivo (Bastian 1999). Assessments
of rheobase for tonic and burst spiking were assessed through a series of 250 ms step
depolarizations until burst threshold was reached (up to 1 nA), with 2 s between pulses.
Baclofen has previously been shown to activate GABA_B receptors in this preparation and
was focally ejected (100 μM) into the PCL using electrodes of 1-2 μm tip diameter and 7-15
psi pressure ejection, as previously described (Berman and Maler 1998b; c; Turner et al.
1994). A visual estimate of the radius of drug application was initially obtained in dendritic
regions of an unrelated region of the slice under transillumination. Previous studies have
estimated a ~10x dilution factor in order to obtain effects consistent with bath application
(Turner et al. 1994; Turner et al. 1991). Pharmacological agents were dissolved in HEPES-
buffered ACSF.
2.2 Data analysis

All electrophysiological data was analysed in Matlab. FI curves and analyses of spike parameters were generated using custom software in MatLab. Spike threshold was taken based on the first derivative of the voltage waveform, and the gain of FI curves were calculated from the linear fit between rheobase and the point of saturation in spike frequency. The size of the AHP was defined as the difference in voltage between the threshold point and the minimum voltage of the AHP. The optimal linear discriminant was calculated according to previously established methods (Metzner et al. 1998), and all bursts, isolated spikes or null triggering events were projected onto this optimal feature $f$. The resulting probability distributions were used to calculate the ROC. The coding fraction $\gamma$ was calculated as:

\[
\text{Eq. 1 } \gamma = (1-\varepsilon),
\]

where $\varepsilon$ is the error in the reconstructed stimulus (Gabbiani et al. 1996).

Spike trains were partitioned into bursts and isolated spikes using an ISI histogram method and ISI discrimination criterion of 8 ms, in agreement with previous work which established that these ISIs are associated with the conditional backpropagation of dendritic spikes that characterize bursting (Lemon and Turner 2000). This is consistent with previous studies examining in vitro time-varying inputs, as burst ISIs can be readily defined using this value given the consistency of burst output in vitro (Oswald et al. 2004). Bursts and isolated spike trains were digitized into binary trains and their means subtracted.
$P_{ss}(f)$

$P_{sr}(f)$

$C(f) = \frac{P_{sr}(f)^2}{P_s(f)P_r(f)}$

Stimulus

Neuron

Spike Train

Binary Spike Train

Coherence

Frequency
Figure 2-1 The calculation of stimulus-response coherence estimates.

A Gaussian white noise stimulus is low-pass filtered (60 Hz), and this time series is used to drive a neuron. The resulting spike train is then digitized, and the power spectrum can be estimated for the original stimulus ($P_{ss}$), the resulting spike train ($P_{rr}$), and the cross-spectrum between the stimulus and response ($P_{sr}$). This can be used to calculate the stimulus-response coherence estimate ($C_{sr}$), which shows the degree to which the frequency content of the stimulus is linearly encoded by the spike train.
(Rieke 1997). Coherence estimates between the digitized spike trains and the original RAM stimulus were calculated as:

$$C(f) = \frac{P_{sr}(f)^2}{P_{ss}(f)P_{rr}(f)}$$

where $P_{ss}$ and $P_{rr}$ denote the power spectrum of the stimulus and the response, and $P_{sr}$ denotes the cross-spectrum between the stimulus and response (e.g. the spike train) and $f$ is frequency measured in Hz. The calculation of the stimulus-response coherence is diagrammed in Figure 2-1. Statistical significance was determined using paired t-tests unless otherwise noted and expressed as mean ± SEM. Statistical significance for post-hoc multiple comparisons after ANOVA was determined using Tukey’s HSD. Nonparametric analyses in Chapter 6 were performed using the Kruskal-Wallis test, followed by individual Mann-Whitney U tests.

2.3 Modelling

2.3.1 Large compartmental model

In Chapter 3 we used a previously published multi-compartmental reconstruction of an ELL pyramidal cell (Doiron et al. 2001b) to test if it could replicate our experimental results. Simulations using this large model were run in the NEURON simulation environment (Hines and Carnevale 1997). In order to examine the effects of the active
dendrite on gain in the most generalizable fashion, the burst dynamics specific to these cells were removed. The initial FI curve was refit to allow for a greater multiplicative effect of the DAP in accordance with our dendritic TTX results. A full summary of all changes is presented in Table 1. Note that the Kv3 current had been functionally removed for this series of experiments. This current was originally based on kinetics of Kv3.3 channels measured in an expression system (Doiron et al. 2001b). We found that the small active repolarization in the dendrites invoked with these parameters prevented the leak conductance from appropriately replicating our experimental results. Because Kv3 channel kinetics and voltage-dependence differ significantly between expression systems and those in situ (Baranauskas et al. 2003), this current was functionally removed by setting the parameter to a sufficiently small level as to be ineffective.

For simulations of synaptic bombardment, 250 excitatory synapses were placed on the terminal bush of the single basilar dendrite, the primary site of afferent input to these cells (Maler et al. 1981). All synapses were described by alpha functions and driven by independent Poisson processes as previously described (Doiron et al. 2001a). Excitatory synapses had a unitary conductance of 300 pS, a time constant ($\tau$) of 1.5 ms, and a reversal potential ($E_{rev}$) of 0 mV (Berman and Maler 1999). One hundred inhibitory synapses (unitary conductance = 25 pS, $\tau$=7ms, $E_{rev}$ = -70 mV) were placed along the apical dendrite in a graded fashion, such that less than 5% of the synapses contacted the 25 $\mu$m most proximal to the soma (Table 2 gives the gradient used for these simulations). These synapses are intended to represent the activity of VML cells and are in accordance with the pattern of their synaptic projections (Maler et al. 1981). The same graded distribution of conductances
was used in distributing tonic leak conductances \( (E_{\text{rev}} = -70 \text{ mV}) \), since the VML cell is the primary source of GABAergic synaptic input onto pyramidal cell proximal apical dendrites. For simulation of GC2 granule cell inhibition of pyramidal somata, we used a larger number of synaptic inputs (200-400) stimulated at 100 Hz. The larger number of synapses was required for any noticeable effect to be observed during somatic inhibition, and represents the greater GABAergic innervation of the soma (Maler and Mugnaini 1994).

### 2.3.2 LIF model

We used the simple Leaky-Integrate-and Fire (LIF) model (Koch 1999; Lapicque 1907) to further study the mechanics of dendrite-dependent divisive gain control in Chapter 5. LIF model neurons have a subthreshold membrane potential \( V_m \) that evolves according to:

\[
\text{Eq. 3} \quad C \frac{dV_m}{dt} = g(E_r - V_m) + I, 
\]

where \( g \) is a passive membrane leak conductance for non-specific leak channels with a reversal potential \( E_r \), \( C \) is the membrane capacitance, and \( I \) is the static input current. Equation (3) is supplemented with a firing rule: when \( V_m(t) = V_t \) a voltage spike is assumed to have occurred at time \( t \). After the spike the membrane is reset to a potential \( V_r < V_t \). The firing rate \( f \) of the model neuron is then simply the inverse of the time \( T \) that it takes \( V_m \) to raise from \( V_r \) to \( V_t \). Explicitly solving eq. (3), \( T \) is determined to be:

\[
\text{Eq. 4} \quad T = \frac{C}{g} \ln \left[ \frac{g(V_r - E_r) - I}{g(V_t - E_r) - I} \right].
\]
The model parameters that best mimicked ELL pyramidal cell firing rates were $C = 150$ pF, $E_r = -70$ mV, $V_t = -55$ mV. $V_r$ varied between -70 and -85 mV while $g$ varied between 5-11 nS. With these parameters the membrane time constant was between 13.6 and 30 ms; values within the range reported for ELL pyramidal cells in vitro (Berman and Maler 1999; Turner et al. 1994). The qualitative model results presented were not sensitive to these parameters.

2.3.3 Reduced compartmental model

As well as the larger multicompartmental model, a reduced model was used to allow non-linear dynamical analysis and to incorporate our most recent results on the biophysical implementation of the burst mechanism. This model consisted of a reduction of an ELL pyramidal cell to two compartments (soma and dendrite), an approach that had previously been used to describe bursting in this system (Fernandez et al. 2005b).

Simulations were constructed in MatLab 7 using a 4th order Runge-Kutta algorithm with a time step ($dt$) of 0.005 ms. All bifurcation analyses were done using the XPP-AUTO package (Doedel 1981; Ermentrout 2002). Our model was described by the following equations:

\begin{align*}
\text{Eq. 5 } m_x &= \frac{1}{1 + e^{-(V + 40)/3}}, \hspace{1cm} \text{somatic Na}^+ \text{ activation} \\
\text{Eq. 6 } \frac{dh_s}{dt} &= \frac{-h_s + h_{s,\infty}(V)}{\tau_{h_s}(V)}, \hspace{1cm} \text{somatic Na}^+ \text{ inactivation}
\end{align*}
Eq. 7 $h_{s\infty} = \frac{1}{1 + e^{(V+40)/3}}$, somatic Na\(^+\) steady-state inactivation and K\(^+\) activation

Eq. 8 $\frac{dm_d}{dt} = -\frac{m_d + m_{d\infty}(V)}{\tau_{m_d}(V)}$, dendritic Na\(^+\) activation

Eq. 9 $m_{d\infty} = \frac{1}{1 + e^{-(V+46.7)/5.7}}$, dendritic steady-state Na\(^+\) activation

Eq. 10 $\frac{dh_d}{dt} = -\frac{h_d + h_{d\infty}(V)}{\tau_{h_d}(V)}$, dendritic Na\(^+\) inactivation

Eq. 11 $h_{d\infty} = \frac{1}{1 + e^{-(V+55)/3}}$, dendritic steady-state Na\(^+\) inactivation

Eq. 12 $\frac{dn_d}{dt} = -\frac{n_d + n_{d\infty}(V)}{\tau_{n_d}(V)}$, dendritic K\(^+\) activation

Eq. 13 $n_{d\infty} = \frac{1}{1 + e^{-(V+12.5)/8.75}}$, dendritic steady-state K\(^+\) activation

All time constants in the model were voltage-dependent and described by a Lorentzian function as used in a previous study (Fernandez et al. 2005a):

Eq. 14 $\tau(V) = y_0 + \frac{2Aw}{4\pi(V-V_c)^2 + w^2}$,

for $h_s$: $V_c = -64$, $w = 28$, $A = 232$, $y_0 = 0$,

for $m_d$: $V_c = -45.7$, $w = 26$, $A = 7.4$, $y_0 = 0$,

for $h_d$: $V_c = -60$, $w = 43$, $A = 301.6$, $y_0 = 0$,
for \( n_d : V_s = -40, w = 30, A = 70, y_0 = 0.4 \),

Voltage in the somatic \((V_s)\) and dendritic \((V_d)\) compartments was integrated according to:

\[
\text{Eq. 15} \quad C_s \frac{dV_s}{dt} = \left( V_d - V_s \right) + I_E - g_{Na_s} m^3 h_s (V_s - E_{Na_s}) - g_{K_s} (1 - h_s)^4 (V_s - E_{K_s}) - g_{leak_s} (V_s - E_{leak_s})
\]

\[
\text{Eq. 16} \quad C_d \frac{dV_d}{dt} = \left( V_d - V_s \right) + \frac{g_{Na_d} m^3 h_d (V_d - E_{Na_d}) - g_{K_d} n^4_d (V_d - E_{K_d})}{(1 - \kappa)R} - g_{leak_d} (V_d - E_{leak_d})
\]

Constants in the somatic and dendritic compartments consisted of the following:

\[ C_s = 1.2 \mu\text{F/cm}^2, \quad C_d = 3.5 \mu\text{F/cm}^2, \quad R = 2/3 \text{k}\Omega/\text{cm}^2, \quad \kappa = 0.35, \quad E_{Na_s} = 40 \text{mV}, \quad E_{K_s} = -88.5 \text{mV}, \quad E_{leak_s} = -72 \text{mV}, \quad g_{Na_s} = 60 \text{mS/cm}^2, \quad g_{K_s} = 12 \text{mS/cm}^2, \quad g_{leak_s} = 0.18 \text{mS/cm}^2, \quad g_{Na_d} = 20 \text{mS/cm}^2, \quad g_{K_d} = 8 \text{mS/cm}^2, \quad g_{leak_d} = 0.18 \text{mS/cm}^2, \quad g_{sGABA} = 0 \text{mS/cm}^2, \quad g_{dGABA} = 0 \text{mS/cm}^2 \]

mS/cm^2 unless otherwise noted. The GABA-like conductances in the soma and dendrite are denoted as \(g_{sGABA}\) and \(g_{dGABA}\) respectively, with the associated reversals of -70 mV for GABA\text{A} and -88.5 mV for GABA\text{B} (Berman and Maler 1998a; b; c). The cell was driven with an external current source \((I_E)\). The reduced model is summarized briefly in Figure 2-2A.

The parameter ‘\(\kappa\)’ denotes the relative contribution of one compartment to the other, with a \(\kappa\) value of 0.35 signifying that the dendritic current influence on the soma is greater than the somatic current influence on the dendrite. The ‘\(R\)’ parameter denotes the resistance between the two compartments. The \(\kappa\) and \(R\) values are similar to those of previous modeling studies using this approach (Doiron et al. 2002; Mainen and Sejnowski 1996; Pinsky and Rinzel 1994; Wang 1999).
Figure 2-2  Diagram of reduced compartmental model and network model.

A) Schematic of single unit model showing the somatic and dendritic distribution of synaptic and voltage gated currents. B) Schematic of network model of the direct feedback pathway between the nP and ELL. Briefly, the ELL excites the nPd, which gives diffuse inhibitory feedback to all ELL pyramidal cells. Each observed spike in ELL pyramidal cells is fed through a feedback kernel representing the nPd. This in turn invokes a synaptic waveform inhibition of pyramidal cells after a delay ($\tau$). Grey lines denote excitatory projections and black lines inhibitory projections. Dendritic compartments receive GABA_B mediated inhibition, while somatic compartments receive GABA_A mediated inhibition (Mehaffey et al. 2007).
2.3.4 Network models

The parameters for network simulations were adapted from Doiron et al. (Doiron et al. 2003a). The network consisted of 100 of the two compartment cells described above, receiving GABA_A mediated inhibition somatically and GABA_B mediated inhibition dendritically (Figure 2-2B). Briefly, the network dynamics were such that they followed stochastic delay differential equations:

\[ \frac{dV_s(i)}{dt} = \frac{(V_d(i) - V_s(i))}{\kappa R} + I - g_{Na} \, m_x^3 h_s(i) (V_s(i) - E_{Na}^-) - g_{Ks} \, (1 - h_s(i))^4 (V_s(i) - E_{K^+}) + g_{sGABA_B} (V_s(i) - E_{Cl^-}) - g_{leaks} (V_s(i) - E_{leak}) + \eta(t) \]

\[ \frac{dV_d(i)}{dt} = \frac{(V_s(i) - V_d(i))}{\kappa R} + I - g_{Na} \, m_x^3 h_d(i) (V_d(i) - E_{Na}^-) - g_{Kd} \, (1 - h_d(i))^4 (V_d(i) - E_{K^+}) + g_{dGABA_B} (V_s(i) - E_{K^+}) - g_{leakd} (V_d(i) - E_{leak}) + \eta(t) \]

\[ \frac{d\eta(t)}{dt} = -\frac{\eta(t)}{\tau} + \sqrt{\frac{D}{\tau}} \xi(t) \]

when a synaptic event (GABA_A or GABA_B) is triggered, it obeys

\[ g_{\text{gaba}_x} = \frac{g_{\text{syn}_x}}{N} \sum_{i=1}^{N} \sum_{m=1}^{M_x} e^{(t-t_{m})/\tau_1} - e^{(t-t_{m})/\tau_1/(\tau_1-\tau_2)} \]

where \( \eta \) represents a Ornstein-Uhlenbeck white noise process (OU Noise), with time constant \( \tau = 14 \) ms, and \( \xi \) represents a noise term. The delay time for feedback was set to 15 ms and the two time constants \( \tau_{GABA_A1} \) and \( \tau_{GABA_A2} \) for the synaptic dynamics of GABA_A.
were set to 3 and 5 ms, respectively, to mimic GABA_A mediated currents in this system (Berman and Maler 1998c). GABA_B mediated currents were modeled more slowly with \( \tau_{GABA_B1} \) and \( \tau_{GABA_B2} \) of 500 ms, again mimicking observed currents in the system (Berman and Maler 1998c; Mehaffey et al. 2007). The GABA_A mediated feedback is triggered in response to each spike of each cell in the network, while the GABA_B mediated feedback is triggered when mean firing rate of the network over a 5 ms period exceeds 50 Hz/cell in order to represent the frequency-dependent activation of GABA_B receptors in this system (Berman and Maler 1998c; Mehaffey et al. 2007). This network is schematized in Figure 2-2B. The time constants and parameters as those described above for the individual cell models. The parameter for the GABA_A mediated synaptic coupling in the network was \( g_{synGABA_A} = 0.185 \mu S/\mu m^2 \), and one synaptic event was triggered for each presynaptic spike. Firing times of cells were sampled at 0.5 kHz. Input current amplitudes were chosen to drive cells at 19-20 Hz (Uncorrelated inputs without GABA_B: 20.17 Hz, Uncorrelated inputs with GABA_B: 19.95 Hz, Correlated inputs without GABA_B: 19.78 Hz, Correlated inputs without GABA_B: 19.37 Hz).

During stimulation, either one cell was driven with known RAM stimuli (0-60 Hz filtered Gaussian white noise (GWN)), while the remainder were driven with an OU process with an equivalent power spectrum (Uncorrelated inputs). In other simulations all cells were driven with the identical known RAM (Correlated inputs). Simulations included parameter sets where \( g_{synGABA_B} = 0.0 \mu S/\mu m^2 \) (e.g, no GABA_B feedback condition), or where \( g_{synGABA_B} = 0.24 \mu S/\mu m^2 \) (e.g with GABA_B mediated feedback).
<table>
<thead>
<tr>
<th>Compartment</th>
<th>Current</th>
<th>Conductance (S/cm²)</th>
<th>Original Conductance (S/cm²) (Doiron et al. 2001b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma</td>
<td>$G_{Na}$</td>
<td>2.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Soma</td>
<td>$G_{DR}$</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Soma</td>
<td>$k_1$</td>
<td>0.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Proximal Dendrite</td>
<td>$G_{Kv3}$</td>
<td>0.0001</td>
<td>1</td>
</tr>
<tr>
<td>Proximal Dendrite</td>
<td>$G_{Na,D}$</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Proximal Dendrite</td>
<td>$G_{DR,D}$</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 1 Changes to the compartmental model originally described in Doiron et al. (2001b).
Table 2 Distribution of the increased leak conductance or percent of the total number of synaptic contacts used in the large compartmental model.

Note that although much of the increased conductance density is in the final dendritic segments, the radii of dendritic branches decrease with distance, reducing the skew in the total change of conductance in each segment.
3.1 Specific Introduction

Individual neurons integrate synaptic inputs and output action potentials (spikes) to convey information. The ability of neurons to divisively modulate their firing rate, rather than simply summatung inhibitory and excitatory synaptic events, represents a significant increase in complexity for single neuron computation. Division or multiplication of firing rate by synaptic input, originally suggested by Reichardt (Reichardt 1961), has become a key component of many models of neural processing (Salinas and Sejnowski 2001), and is required in models characterizing the response of cells in visual cortex (Carandini and Heeger 1994) and efficient coding in visual and auditory systems (Schwartz and Simoncelli 2001). Early investigations into biophysical mechanisms for division of firing rate were based on Ohm’s law: that membrane potential is equal to current divided by membrane conductance (Blomfield 1974; Koch and Poggio 1992; Nelson 1994). Divisive changes in membrane potential due to increased conductance were assumed to translate to a division of firing rate. Later theoretical studies showed that shunting inhibition is unable to divisively modulate firing rate (Gabbiani et al. 1994; Holt and Koch 1997). Instead, changes in subthreshold membrane conductance led to primarily inhibitory effects on spike threshold, causing a subtractive shift in the onset of firing by changing the rheobase.

Since this time, a number of studies have sought to determine a mechanism that allows synaptic input to regulate the gain of an individual cell. Dynamic clamp (Chance et al. 2002; Mitchell and Silver 2003) and modelling of various configurations of synaptic
input (Burkitt et al. 2003; Doiron et al. 2001a; Longtin et al. 2002; Prescott and De Koninck 2003; Salinas and Sejnowski 2000; Tiesinga et al. 2000) have shown that noise can generate divisive effects. These studies indicate that after balancing of stochastic inputs, FI curves have a stationary current threshold for initial spiking (rheobase), while the gain (in Hz/nA) decreases. However, studies of noise-induced gain have used the variance in membrane fluctuation to drive spiking in a probabilistic fashion. This suggests that as the time window used to calculate average spike rate is increased, the likelihood of successfully encoding transient inputs may be reduced. In a sensory system that depends on correct detection of brief events, such averaging could be disadvantageous.

I describe in this chapter how proximal dendritic inhibition is capable of regulating a dendrosomatic current flow arising from backpropagating dendritic spikes. This gives rise to a novel deterministic regulation of the gain of the spike generating mechanism that is distinct from all previous mechanisms that required balanced, noisy synaptic conductance changes near the spike generating mechanism. The biophysical components required for this dendritic division of neuronal inputs are simple and widespread, and thus may also be utilized in many other systems.

3.2 Results

3.2.1 Divisive gain control is mediated by dendritic but not somatic inhibition

First order electrosensory processing in *A. leptorhyncus* is accomplished by pyramidal cells of the ELL. Appropriate input can modulate the pyramidal cell’s firing
rate from 0-200 Hz (Bastian 1986a). The ELL contains many interneurons that can modify pyramidal cell output (Maler 1979; Maler and Mugnaini 1994), and two GABAergic interneurons are especially notable (see also Chapter 1). Briefly, the GC2 interneuron receives direct electroreceptor input and projects to the pyramidal cell soma. In contrast, the inhibitory VML cells receive descending feedback and project to the region of apical dendrites of pyramidal cells that actively propagate sodium spikes (Figure 3-1A). These inputs are distributed along the proximal dendritic axis, showing a gradient of synaptic density that peaks approximately 100 μm from the soma, and decreases to less than 5% of total contacts near the soma (Maler and Mugnaini 1994; Maler et al. 1981). In both cases the inhibition is mediated by the GABA_A receptor subtype (Berman and Maler 1999). In order to evaluate the role of these interneurons in the regulation of sensory processing, we mimicked their activation by focal application of a selective GABA_A agonist, muscimol. Muscimol (200 μM) was focally applied to either the soma (n = 9) or the proximal dendritic region (n = 14) of pyramidal cells while recording from their somata. Because of the architecture of the ELL, we were able to use the StF fibre bundle that overlies the PCL as a barrier to the diffusion of pharmacological agents, and can therefore separate dendritic and somatic effects of applied modulators on cell output (Turner et al. 1994).

We used a series of 500 ms DC current steps to derive FI plots, and the frequency of firing was averaged over the course of the current pulse irrespective of discharge pattern (both tonic and bursting modes of discharge were observed and the following results were the same for either mode). Pharmacological activation of dendritic GABA_A receptors led to a divisive effect on the FI relationship, with minimal effects on the
rheobase to commence firing (Figure 3-1B). Linear fits from the rheobase to the saturation point of the FI curve revealed a significant mean decrease in slope of 37 % (± 6.3 %, p < 0.0001). This slope represents the gain of the firing system in Hz/nA. The rheobase for initial spiking did not change significantly, with a mean shift of 0.08 nA (± 0.07 nA, p >0.15). The majority (n = 9) of these cells showed no, or only minor subtractive effects (less than 0.03 nA), while one showed very large subtractive effects (0.8 nA), possibly due to muscimol leaking to the somatic region over the course of the experiments (see below). To ensure divisive effects were not due to the diffusion of muscimol during the recording of individual FI curves, repeated recordings were made after the dendritic muscimol ejection. During drug diffusion, the divisive effects described were reproducible between multiple FI recordings. This indicated that the changes within a single FI curve were not due to a progressive diffusion of the drug over the course of recording. Rather it was due to the continuous activation of GABA<sub>A</sub> mediated conductances in the dendritic region over the entire course of the recording.

In contrast, somatic application of muscimol (n = 3) had subtractive effects on FI curves (Figure 3-1C). Sequential application of muscimol to both the soma and dendrite (n = 6) yielded identical results, with no further reduction in gain (Figure 3-1D). As no differences were noted between the two conditions the data sets were merged. These experiments showed that somatic muscimol application did not lead to a significant reduction in slope of the FI relationship (5.4 % ± 6.5%, p > 0.45), but did result in a significant shift in rheobase (0.59 nA ± 0.16 nA, p < 0.01). In a further two cells, the resulting shift in rheobase was sufficient to prevent firing in response to more than 1 nA.
Figure 3-1 Dendritic inhibitory conductances modulate gain divisively, while somatic inhibitory conductances are subtractive.

A) Simplified architecture of the ELL indirect feedback pathway. Parallel fibre feedback of the EGp cerebellar granule cells drive both the pyramidal cell (black) and the VML cell (grey). The VML cell gives rise to inhibitory synapses contacting the apical dendrites of pyramidal cells. Inhibitory inputs to the pyramidal cell soma come from many cells including GABAergic GC2 cells and nP bipolar cells (Berman and Maler 1999). B) Focal application of the GABA\textsubscript{A} agonist muscimol (200 \textmu M) to the apical dendrites (\textgradient, dashed line) causes a divisive shift in the FI relationship relative to control (■, solid line) without significantly affecting the rheobase to spiking. C) Application of muscimol to the soma subsequent to dendritic ejection (\textgradient, dashed line) fails to further divide the FI curve obtained after dendritic muscimol (■, solid line), having instead a subtractive effect. D) Application of muscimol to the soma alone in a separate recording does not shift the slope of the FI curve (\textgradient, dashed line), but shifts the spike rheobase relative to control conditions (■, solid line). Lines in B and C are linear fits to the FI plot between rheobase and peak firing frequency. All FI plots have been normalized such that control rheobase is zero.
of current injection above the previous rheobase. These cells were not included in the analyses of slope or rheobase, since no spiking was observed.

These differential effects of muscimol would suggest distinct computational properties of dendritic versus somatic inhibition in our cells: dendritic inhibition has a divisive effect while somatic inhibition has subtractive effects, in confirmation of Holt and Koch (1997).

### 3.2.2 The DAP has an inherently multiplicative effect

In the ELL pyramidal cell, dendritic sodium channels underlie an active backpropagation of the somatic spike through the initial portion of the apical dendritic tree (Lemon and Turner 2000; Turner et al. 1994). These active dendritic conductances result in current flow back to the soma, and can create a DAP (Figure 3-2A,B). We first examined the contribution of dendrosomatic current flow to the gain of the firing response. Since DAPs arise from active regions of the dendrite that the VML cell selectively inhibits, we further examined the role of dendritic inhibition in modulating gain. We blocked active dendritic conductances by focal application of tetrodotoxin (TTX) to the proximal dendritic region (n = 6). We monitored these recordings in order to ensure that TTX was restricted to the dendrites and had not leaked to the soma. No significant decrease in the peak amplitude or rate of rise of the somatic spike was observed (Figure 3-2F,G). Dendritic application of TTX revealed a large somatic AHP which had previously been masked by the DAP (Figure 3-2C,F), while the FI curve showed a marked reduction in gain (Figure 3-2H). This suggests that a significant dendrosomatic current flow exists even when a prominent DAP cannot be observed in the
Figure 3-2 Depolarizing afterpotentials multiplicatively affect the gain of the FI response.

A) Schematic diagram of DAP generation by active dendrites- a somatic spike (1) backpropagates into an active dendrite (2), creating a voltage difference between the compartments that leads to dendro-somatic current flow, producing a DAP (3). B) Superimposition of a somatic (black) and a dendritic (grey) spike from separate recordings to show the relationship between the dendritic spike and the DAP. C) Blockade of dendritic sodium channels by application of TTX to the dendrites during somatic recordings removes the DAP and unmasks a large AHP (grey trace) relative to control conditions (black trace), without affecting the height or the shape of the somatic spike. D) Somatic and dendritic spikes in a compartmental model replicate the essential features of spiking seen in the pyramidal cell. E) Masking of the fast AHP by dendro-somatic current flow due to backpropagating dendritic spikes in the compartmental model. A large AHP is visible in the case without dendritic sodium (grey), and is partially occluded when active dendritic conductances are included (black). F) Expanded view of (C) showing no change in the rate of rise or height of the somatic spike after dendritic TTX ejection. G) Peak amplitude and rate of rise of the somatic spike in 6 separate experiments are unaffected by dendritic TTX ejection, indicating a specific dendritic effect of TTX. H) Modulation of the FI relationship by active dendritic conductances. Blockade of active dendritic conductances by dendritic application of TTX (○) leads to a much shallower gain in response to step current injections when compared to control conditions (■). I) The model illustrates the insertion of conductances generating active
dendritic backpropagation leads to a dramatically steeper FI relationship (■) compared to a passive dendritic model (○).
We next sought to replicate these effects in a large multi-compartmental model of an ELL pyramidal neuron (see Methods, Chapter 2), where passive dendrites produced a shallow F-I curve. The addition of dendritic Na⁺ conductances was sufficient to reproduce an appropriate dendritic spike waveform (Figure 3-2D), which masked a large portion of the somatic AHP (Figure 3-2E) and raised the slope of the FI relationship (Figure 3-2I). These results show that the DAP, a common product of active dendrites, has an inherently multiplicative effect on the FI relationship. Intuitively this can be understood through a consideration of the dependence of the feedback on the generation of spikes. Specifically, the amount of dendrosomatic current flow scales with firing rate, while the firing rate also scales with the amount of dendrosomatic current. The fact that the DAP requires initiation of at least one spike forces the rheobase to be invariant with respect to the injected current. Importantly, this inherent change in firing frequency should occur under any regime where a backpropagating dendritic spike follows a somatic spike, regardless of their exact shapes. Furthermore, the results obtained from muscimol application (Figure 3-1) suggest that this multiplicative effect can be diluted by dendritic inhibitory conductances.

3.2.3 The contribution of the DAP can be regulated by dendritic leak conductances

In our system, and in many others, the DAP depends on active dendritic conductances (Larkum et al. 1996; Magee and Carruth 1999; Turner et al. 1994), although dendritic spikes typically have a slower process of repolarization and weak AHPs (Reyes 2001). Since we suspected that regulation of active dendritic conductances
was involved in modulation of gain, recordings were made from the proximal dendrites of pyramidal cells during focal dendritic application of muscimol (n = 5). Muscimol ejection selectively changed the repolarization phase of dendritic spikes, allowing for a faster decay at the end of the spike (Figure 3-3A, muscimol trace). These changes in the dendritic spike were seen in separate somatic recordings as a small reduction in the DAP, manifesting itself as a larger AHP following repolarization (Figure 3-3B). Analysis of the AHPs recorded somatically following dendritic muscimol ejection revealed that under control conditions, the mean AHP size was 4.85 mV (± 0.95 mV), while after tonic activation of dendritic GABA_A synapses, the mean AHP size increased to 7.3 mV (± 1.67 mV), representing a 35% increase in the AHP (± 4.6%, p < 0.0001).

In contrast, ejections of muscimol to the cell body layer did not create a significant change in AHP size. Under control conditions, the mean AHP size for this population was 6.3 mV, while after ejection of somatic muscimol the mean AHP was 6.65 mV, giving an average increase of 4.7 % (p > 0.74). As described above, somatic muscimol ejection did not produce divisive effects, but did cause a subtractive change in rheobase for the FI curve. This indicates that dendritic, but not somatic, muscimol application affects the width of the dendritic spike and subsequently, the size of the somatic AHP. Reductions in dendritic spike width are tied to an increase in the size of the somatic AHP through a reduction in the DAP, which would normally partially mask the fast AHP. Therefore regulation of the AHP by inhibition of DAP amplitude is a property exclusive to the apical dendrites of the ELL pyramidal cell.

Replication of the muscimol experiments in a compartmental model led to similar results. Increasing the dendritic leak to simulate GABA_A receptor activation led to a
reduction in spike width during the final decay of the dendritic spike, and a slightly lower interspike voltage (Figure 3-3C). This dendritic change manifested itself as a change in the depth of the somatic AHP (Figure 3-1D). This modest change in dendritic spike properties and somatic AHP proved to be sufficient to reproduce the changes in gain associated with the dendritic muscimol application, enabling a fast regulation of the gain. Furthermore, dendritic spike width predicted the gain in the firing model.

Specifically, by increasing the total density of a Cl⁻ leak conductance in the dendrites, we were able to significantly shift the gain of the modelled FI relationship with minimal effects on spike rheobase (Figure 3-3E, Figure 3-1B). In comparison, increases in the density of the somatic leak conductance had no change on the gain of the system, but instead acted in a purely subtractive manner (Figure 3-3F, c.f. Figure 3-1C) a phenomenon that has been well established for somatic inhibition (Gabbiani et al. 1994; Holt and Koch 1997). This shows that changes in the somatic AHP, on the same scale as seen in our recordings and induced by changes in dendritic leak in the model, are capable of producing a divisive modulation of the gain.

3.2.4 Reduced model of divisive computation

Both the experiments and large-scale compartmental modeling suggest that a dendrite capable of action potential backpropagation is a key requirement for this mechanism of gain control. Dendritic TTX results in a larger somatic AHP since the DAP and AHP overlap in time following a somatic spike, allowing the DAP to mask a fast AHP. An expanded view of the repolarization phase of a somatic action potential shows that a larger somatic AHP results
Pyramidal Cell

A

- Control
- Dendritic Muscimol

20mV
4ms

Dendrite
Soma

Model

C

- Control
- Dendritic Leak

20mV
5ms

Dendrite
Soma

E
Dendritic Leak (Model)

F
Somatic Leak (Model)

Frequency (Hz)

0
20
40
60
80
100
120

0
0.1
0.2
0.3
0.4
0.5
0.6

Current (nA)

0
20
40
60
80
100

0
0.1
0.2
0.3
0.4
0.5
0.6

Current (nA)

- 0.2 uS/cm²
- 0.6 uS/cm² Gik
- 1 uS/cm² Gik

- 0.25 uS/cm²
- 5 uS/cm² Gik
- 25 uS/cm² Gik
Figure 3-3  A reduction of the DAP and corresponding increase in AHP divide the FI relationship.

A) Application of muscimol to the apical dendrites while recording from dendrites (grey trace) reveals a selective reduction in width at the base of the dendritic spike. B) This reduction in dendritic spike width is correlated with a larger AHP (grey) relative to control conditions (black) in separate somatic recordings. C) In the compartmental model, the dendritic recording has a faster decay time at its base under “experimental” conditions (increased dendritic $G_{lk}$, grey) as compared to control conditions (black). D) An increase in dendritic leak (0.6 $\mu$S/cm²) leads to a larger AHP at the soma (grey) relative to the control model (black). E) Dendritic leak is sufficient in the model to replicate the gain changes seen experimentally after GABA$_A$ receptor activation in pyramidal cell recordings. Increasing levels of dendritic leak (3x (▲) and 5x (○)) cause divisive effects in the FI relationship compared to control (■). F) In contrast, increasing the somatic leak by 10x (▲) or 20x (○) causes purely subtractive shifts without noticeably affecting gain relative to control conditions (■)
from dendritic TTX application, while a similar effect can also be seen in the large compartmental model of a pyramidal cell (Figure 3-4A, left and center traces). In this section we use a simple Leaky-Integrate-and-Fire (LIF) model to show how the DAP influence on the AHP is the crucial feature of this mechanism of gain division, and to derive specific rules for the sort of manipulations that can lead to divisive gain control.

To model somatic muscimol application we increased the leak conductance $g$, similar to the strategy used in the large compartmental model. However, standard LIF models lack any formal concept of a spatially extended dendritic tree, making explicit modeling of dendritic muscimol application difficult. Rather than formally incorporating an excitable dendritic membrane dynamic (Larkum et al. 2004) we chose to model and manipulate the effect of dendro-somatic current flow on the somatic AHP. Quite simply we modeled the somatic effect of dendro-somatic current flow as regulating the depth of the “AHP”, i.e. the membrane potential that the LIF is reset to after a spike ($\Delta V_r$; Fig. 4A, right trace). Specifically, a more negative $V_r$ mimics the increase in AHP depth associated with DAP reduction resulting from dendritic muscimol application.

When $g$ was increased (modeling the application of somatic muscimol) we observed a subtractive shift in FI curve gain (Figure 3-4B). This result has been previously observed in simple IF models and is now a well-known effect (Doiron et al. 2001a; Holt and Koch 1997). Figure 3-4C shows FI curves computed for the LIF model for various values of $V_r$ with $g$ fixed in all cases (modeling the application of dendritic muscimol). When $V_r$ was more negative, a marked decrease in FI slope was observed, with no change in the minimal current required for discharge. These results mirror those shown in Figure 3-1 and Figure 3-3 for both the experimental and large-scale simulation
results. Figure 3-4C shows that simple shifts in action potential reset can account for a divisive gain change. Such a relation between gain and $V_r$ has been previously noted for LIF models (Troyer and Miller 1997). However, it has never been interpreted as modeling a variable dendritic feedback to the soma, or any other dynamic process, but has instead been used as a fixed parameter to regulate default gain. With this simple model, we expand on the mechanism to further link the subthreshold dynamics and divisive gain computations.

Specifically, we can define a multiplicative gain change as:

\[ f'(I) = \kappa(I) f(I). \]

where $\kappa$ is the factor scaling the original FI curve. A ‘pure’ multiplication will be one that allows $\kappa(I) = \kappa$ so that $f(I)$ is scaled in an input-independent fashion during a multiplicative computation. Such a specific definition of multiplicative computations allows for the characterization of simple rules determining the regulation of FI relationships.

It is useful to consider the effects of a gain manipulation in the temporal domain rather than the frequency domain, as it appears in Eq. 21, since spike firing occurs over time. Using the relation $T = 1/f$ it is simple to write that any manipulation resulting in a multiplicative scaling of the initial FI curve (as defined in Eq. 21) must satisfy the following relation:

\[ \frac{\Delta T(I)}{T(I)} = \frac{1 - \kappa(I)}{\kappa(I)}. \]
Here \( T(I) \) is the original threshold crossing time (e.g. the default ISI) and \( \Delta T(I) = T'(I) - T(I) \) is a decrease (or increase) in that ISI after the gain manipulation. Eq. 22 gives a rule for any division of an FI relationship, specifically that in order for a gain manipulation to lead to a divisive computation it is required that the original period \( T(I) \), and the change in period \( \Delta T(I) \) scale with each other. Such a rule can be intuitively understood in that to double the frequency of firing at 1 Hz, the period must be advanced by 500 ms, while to double the frequency of firing at 10 Hz requires an advance of 50 ms.

Because a shift in \( V_r \) induced a slope change in the FI curve in the simple LIF model (Figure 3-4C) this manipulation is a good candidate to satisfy Eq. 22. We show this for two values of input current \( I_1 \) (100 pA) and \( I_2 \) (200 pA), and for each input we computed \( \Delta T(I) \) for models with different voltage resets (\( \Delta V_r \)) (Figure 4D,E). Naturally, as input current increases, the ISI decreases. Interestingly, the effects of \( \Delta V_r \) grow with \( T \). The resulting effect on the FI curve is a divisive gain change, indicating that to some approximation our system obeys Eq. 22, consistent with the apparent scaling of the FI relationship.

Because our simple LIF system obeys Eq. (2) with \( \Delta T(I) \) resulting from \( \Delta V_r \), we can combine the left hand side of Eq. 22 and the LIF model (Eq. M2) and isolate for \( \kappa(I) \) (first equality in Eq. (3)). We next consider system parameters that ensure \( g(V_r - E_r)/I \), \( g(V_r - E_r)/I \), and \( g(V_t - E_r)/I \ll 1 \). Specifically, we have these terms ranging from near zero to 0.5 for the parameters used in Figure 3-4. We compute the Taylor series expansion of \( h(V_r, I) \) and \( h(V_r', I) \) using \( \ln(1-x) \approx -x - x^2/2 \) for small \( x \) (second equality in Eq. (3)).
\[ \kappa(I) = \frac{h(V_r, I)}{h(V_r', I)} \approx \kappa_0 \left( 1 - \frac{g(\Delta V_r)}{1 + g(V_r + V_r' - 2E_r)/I} \right); \]

where \[ h(V, I) = \ln \left( \frac{1 - \frac{g}{I}(V - E_r)}{1 - \frac{g}{I}(V - E_r)} \right), \]

and where \( \kappa_0 = (V_r - V_r')/(V_r - V_r) \) is the relative change in reset voltage as compared to the spike threshold. The expansion shows that although the gain change appears multiplicative (Figure 3-4C), the neuron model can only exhibit a perfect division of firing rate in the limit where conductance \( (g) \) approaches zero.

Figure 3-4F shows \( f(I) \) and \( f'(I) \) computed from the LIF model with a 3 mV change in reset voltage. We also plot \( f(I) \) multiplied by the zero\(^{th}\) order approximation \( \kappa_0 \), valid in the limit of zero input conductance. The approximation \( \kappa_0 \) roughly describes the divisive computation for the parameters used in our model.

In our \textit{in vitro} experiments, an increase in AHP amplitude led to a change in gain, but these two factors were not linearly correlated. This is predicted from our above results, since ELL pyramidal cells have low input resistances (40-100 M\( \Omega \)), meaning \( \kappa \) will be a complex function of input current and membrane conductance. Therefore the range of currents injected and the resting membrane conductance of each cell will have significant effects on attempts to predict \( \kappa \) based on \textit{in vitro} experiments. The above results also show that it is unlikely that a perfect division, where the firing rate is equally scaled
Figure 3-4 Divisive and subtractive effects can be replicated in a LIF model, allowing for analysis of multiplicative computation.

A) Representative changes in the somatic AHP due to active dendritic conductances in the real cell, and in the compartmental model compared to the reduced LIF model. Gray lines indicate manipulations removing dendritic sodium conductances (left, center) or a change in $V_r$ (right). B) Changes in conductance of the LIF model lead to subtractive shifts in rheobase. As conductance ($g$) increases, rheobase changes, but no change is seen in gain. C) Changes in the reset voltage $V_r$, simulating regulation of the AHP size by dendritic conductances, lead to divisive changes in gain. As $V_r$ changes, the gain of the LIF cell can be increased or decreased. D,E) For two values of $I$, the change in ISI ($\Delta T$) resulting from a change in AHP ($\Delta V_r$) scales with the unaltered ISI ($T$). Specifically, for $I_1 = 100$ pA (D) the initial ISI ($T_i$) of the cell is longer than the ISI for $I_2 = 200$ pA ($T_2$). Importantly the threshold crossing delay due to the perturbation $\Delta V_r$ when $I = 100$ pA ($\Delta T_1$) (D) is larger than the delay due to the identical perturbation when $I = 200$ pA ($\Delta T_2$) (E). Such scaling of $\Delta T$ with $T$ shown (D,E) is required for $\Delta V_r$ to induce a divisive effect on the FI curve (Eq. (3), Results). F) The change in gain due to the perturbation $\Delta V_r$ is imperfectly described by a single scaling factor $\kappa_0$ (solid black line) where $\kappa$ is independent of the input current $I$. 
across the entire range of driving currents, will occur. Since cells with large dendritic
arborizations have large input conductances, the scaling of the gain in many cells is likely
to be uneven across the FI plot. Although most models involving division of firing rate
assume a pure division (Carandini and Heeger 1994; Schwartz and Simoncelli 2001), a
quasi-divisive effect, as seen in our data, will also accomplish this and may even allow
for more complex calculations.

We define a simple rule defining the requirements that must be satisfied by any
candidate deterministic gain control mechanism (Eq. 22). This was then compared to the
behaviour of the LIF system. Since the change in reset voltage in the LIF satisfied this
rule, and replicated the effects seen in our data and compartmental model, and since
prominent DAPs were absent in many of our cells and our compartmental model, we
conclude that control of the AHP by the regulation of active dendritic conductances is the
key variable defining this gain control mechanism. This AHP scaling mechanism
functions through the fundamental integrative properties of the membrane, allowing the
time to threshold to vary with the effectiveness of the AHP-mediated perturbation (Figure
3-1).

3.2.5 Divisive gain control by unbalanced synaptic bombardment

The gain control mechanism shown above functions independently of noise.
However, noise is an important component of neural systems, and is key to the
functioning of all previous mechanisms which have successfully controlled gain in a
single cell (Chance et al. 2002; Doiron et al. 2001a; Mitchell and Silver 2003; Prescott
and De Koninck 2003). To examine the interactions between our mechanism and synaptic noise, we simulated stochastic synaptic inputs in order to examine the more realistic noisy scenario. We distributed 250 excitatory synapses along the basal bush of the pyramidal cell model, consistent with morphological data (L Maler, unpublished observation) and a previous study of noise-induced gain control in this model (Longtin et al. 2002), giving us a more realistic driving current than somatic current injection. Inhibition by the VML cell was simulated with 100 inhibitory GABA_A synapses distributed in a graded fashion along the apical dendritic tree, such that less than 5% were proximal to the soma (Maler et al. 1981). We drove the model cell with a range of excitatory synaptic input frequencies combined with dendritic inhibitory synapses firing at 100 or 200 Hz (the maximum firing frequency of VML cells in vitro; unpublished observation). This led to a division of the FI curve that increased with the frequency of inhibitory stimulation (Figure 3-5A,B). Note that these results mirror the divisive effects that were induced by a deterministic shift in dendritic leak conductance (Figure 3-5E).

We can therefore conclude that unbalanced random dendritic inhibition is capable of the same divisive computation seen in our previous experiments. Further, the division still functions in the more natural scenario where the excitation is dendritic in origin, rather than injected somatically.

To simulate the greater number of sources of somatic inhibition existing in the ELL (Maler and Mugnaini 1994), we attached more inhibitory synapses to the somatic compartment. Somatic inhibition, despite the slight increase in stochastic forcing due to the noisy inhibitory conductances being placed closer to the site of spike initiation, showed a subtractive computation with apical or basilar dendritic excitation in this noisy
We would like to note that this does not contradict previous studies of noise-induced gain control, which have either required a small number of synaptic inputs paired with somatic inhibition (Mitchell and Silver 2003), or used a combination of dendritic excitation and somatic inhibition and a third current source (Prescott and De Koninck 2003) in order to divisively modulate firing. The differences in the effects of stochastic somatic inhibition between these studies and our own are due solely to these differences in parameters.

These results demonstrate that in a realistic noisy regime for our system, our deterministic method of gain control has a greater effect than noise alone, since the highest noise condition showed the least change in gain. Instead, even under synaptic stimulation conditions, dendritic regulation of the somatic AHP successfully controlled gain without the requirement for synaptic balancing. This provides a mechanism for the division of firing rate without the deleterious effects of noise.

3.3 Discussion

Modulation of gain is of great importance to computations performed by the nervous system (Reichardt 1961; Salinas and Sejnowski 2001). We have shown a deterministic gain control mechanism based on dendritic inhibition. Specifically, a multiplicative effect (e.g. feedback excitation due to the DAP) is scaled down by dendritic inhibition, leading to an ultimate divisive effect. Compartamental modelling and analytics show that this mechanism provides a graded control of the cell’s
Figure 3-5 Stochastic inhibitory synaptic bombardment of the dendrite induces divisive gain control without balancing.

A) The model cell is driven by excitatory inputs to the distal basilar dendrite, combined with inhibitory synaptic inputs to the proximal apical dendrite. This combination leads to a divisive control of gain, in which gain decreases with increasing inhibitory frequency (100 Hz (○), 200 Hz (▲)). Both conditions are divisively modulated relative to control (■). B) When excitatory synaptic drive is moved to the apical dendrite, a subtractive effect occurs in combination with the divisive effects when paired with inhibitory conductances in the dendrite ((100 Hz (○), 200 Hz (▲))). Both conditions are divisively modulated relative to control (■). C,D) In comparison, placing the inhibitory synapses onto the soma (200 (○) and 400 synapses (▲)) leads to a subtractive rather than divisive shift relative to control (■) regardless of the site of excitation. Schematic diagram to the left of each frame indicates a basilar pyramidal cell in relation to the sites of synaptic inhibition or excitation. Dashed lines indicate the fibre bundles that define the regions of the PCL, and the StF which separates the proximal dendritic from the somatic region.
responsiveness, and follows the rules expected for a mechanism of divisive gain.

Although it has been shown that the AHP is capable of regulating default gain (Smith et al. 2002; Troyer and Miller 1997), we show here that feedback from active dendritic conductances can scale the size of the AHP, allowing for fast changes in the gain of the cell. Further, a spatial segregation of inhibitory inputs to distinct compartments allows for a separation of computations: somatic inhibition allows for algebraic subtraction, while dendritic inhibition allows for divisive control of gain.

Previous experiments and modelling studies have shown the ability to obtain divisive gain control through noisy synaptic input using balanced inputs or dendritic saturation in order to maintain a stationary rheobase (Chance et al. 2002; Prescott and De Koninck 2003). However, even small differences in balancing can alter rheobase, as well as gain (Shu et al. 2003). Alternatively, unbalanced excitatory inputs can be sufficient to offset a tonic inhibitory conductance, but this mechanism functions only with limited numbers of excitatory inputs in small cells (Mitchell and Silver 2003). Such balancing is not required in the mechanism described here. Balanced, noisy conductance changes can, and do, have divisive effects on gain, but imperfections in balancing can have significant effects on rheobase. We show a mechanism that is minimally subtractive, since modulation of the gain occurs after spiking has already been initiated. Separation of the site of inhibition from the site of spike initiation also leaves the integrative properties of the somatic membrane unchanged, allowing gain to be modulated with minimal change in either rheobase or filtering properties of the membrane.

A previous study of a neuron of the fly visual system showed divisive regulation of membrane voltage by dendritic inhibition in non-spiking lobular plate tangential cells
(Single et al. 1997), but this would not be expected to carry over to the spiking regime. A model developed to account for multiplicative computations in the locust included dendritic subtraction in the subthreshold domain; multiplication of inputs also required non-linear transformations by spiking conductances (Gabbiani et al. 2002). This model represents an entirely different multiplicative function that does not require backpropagating spikes and DAPs, or control of the gain of the firing mechanism.

Divisive gain control has been explicitly shown *in vivo* in the ELL (Bastian 1986a), and our results may explain some apparently paradoxical observations in this system. Lesion of the indirect descending excitatory parallel fibre tract that terminates in the ELL molecular layers (Figure 3-6A) increased gain multiplicatively (Figure 3-1B,C), despite the primarily excitatory nature of this parallel fibre tract *in vitro* (Berman and Maler 1999; Lewis and Maler 2002) and *in vivo* (Bastian 1998). Previous work has shown this tract to have a significant role in stimulus cancellation on a time scale of seconds, which would not predict a divisive role (Bastian 1996a; b; 1998; Bastian et al. 2004). Instead, we suggest that parallel fibres also excite the VML interneurons that exclusively inhibit the proximal apical dendrites of pyramidal cells (Berman and Maler 1999; Maler et al. 1981). The work presented here suggests that inhibition at this site will divide pyramidal cell output firing rates. Thus, the removal of the excitatory drive to these cells by cerebellar lesions would be expected to eliminate the divisive gain that results from proximal dendritic inhibition and thus explain the multiplicative results observed after lesioning (Bastian 1986a).

It has been suggested that tonic inhibition can have inherent divisive effects due to a non-linear pre-processing of the input, and a power-law voltage-firing rate
Figure 3-6 Divisive gain modulation *in vivo* involves the EGp feedback pathway.

A) Diagram of the lesions performed in Bastian (1986). Lesion of the excitatory pathway projecting from EGp granule cells removes excitatory parallel fibre inputs to both ELL pyramidal and VML cells. B,C) Multiplicative gain changes in pyramidal cell firing after performing the lesions described in (A). Pyramidal cells responding preferentially to either upstrokes (B) or downstrokes (C) in external electric fields communicated by peripheral electroreceptors are more strongly excited at high levels of stimulation after lesion, but firing rate is unchanged at low levels of stimulation. The suggested role of the VML cell in divisive gain control provides an explanation for the apparently paradoxical effects of this manipulation. Data from Bastian (1986).
relationship induced by background noise (Murphy and Miller 2003). Since the divisive gain control effects were reproduced in slice experiments in which all pre-processing is absent, we can therefore discount this mechanism as contributing to the effects we observed. Other models examining gain have included forward-propagating dendritic spikes that are either inhibited (Archie and Mel 2000) or recruited (Larkum et al. 2004). In Archie & Mel (2000), synaptic input triggers forward propagating dendritic spikes and inhibition reduces the rate of these spikes rather than their amplitude, reducing excitatory drive to the cell. Evidence from in vitro studies of neocortical pyramidal cells has shown that the site of current injection along the apical dendrite can influence the gain of firing through the all-or-none recruitment of dendritic spikes (Larkum et al. 2004), and subsequent bursts of activity. Because these dendritic spikes originate independently of the somatic spike, they cannot satisfy the mechanism we describe here. Notably, when noise is absent the gain change is all-or-none, and is associated with a large change in rheobase. Finally, a study of a pyramidal cell compartmental model demonstrated that combining saturating dendritic excitation with noisy somatic shunting inhibition can lead to gain control (Prescott and De Koninck 2003). This form of divisive regulation is stochastic, in contrast to our deterministic method of DAP/AHP scaling. Therefore the alternative dendritic mechanisms summarized above either remain fundamentally stochastic, or do not regulate the gain of the firing mechanism, and are thus distinct from the mechanism described here.
3.3.1 General applicability

There are three basic requirements for our proposed mechanism to work, and any cell with all three should be able to regulate gain via dendritic input. Firstly, an active dendrite that can generate a backpropagating spike. Dendritic spikes appear to always be wider than somatic spikes, with their peaks occurring later (Reyes 2001). This difference in membrane potential between the dendritic and somatic compartments obligatorily creates a brief positive dendrosomatic current, producing some DAP at the soma. Many cell types show strong DAPs (Kang and Kayano 1994; Nunez 1993), easily visible in the membrane voltage trace that have been shown or suggested to be due to active dendritic conductances (Larkum et al. 1996; Magee and Carruth 1999; Turner et al. 1994). It is important to emphasize that although a DAP might not be easily visible in the membrane voltage trace of many cells, active dendritic backpropagation should always provide some degree of dendrosomatic current flow. In many of our traces, the DAPs were not visible in the voltage trace until their contribution was revealed by blockade of active dendritic conductances. Thus, we do not expect that a cell has to exhibit a visually prominent DAP in order to display this kind of gain control, but some contribution of dendritic current from active dendrites is required.

A second requirement is a process to control the size of the DAP. In our case, dendritic inhibition is capable of reducing the contribution of dendritic inward current to the soma. We note that in both CA1 hippocampal pyramidal cells and olfactory bulb mitral cells, dendritic inhibition can reduce the dendritic action potential (and therefore the reciprocal dendrosomatic current flow) (Lowe 2002; Tsubokawa and Ross 1996).
The third requirement for our proposed mechanism is that the response to a DAP is always to advance the timing of the next spike (e.g. a type I membrane, Ermentrout 1996). Interestingly, in some cells an excitatory pulse immediately following spiking can delay the onset of the next spike (Hansel et al. 1993). Such a mechanism would invert the effects of the spike triggered dendro-somatic current flow described here, leading to inherent divisive effects whose reduction by dendritic inhibition would cause a net multiplicative effect. The simplicity of this gain control mechanism is demonstrated by the reproduction of the key effect by a LIF model (Figure 3-4). This guarantees that the mechanism does not depend on the complicated assortment of nonlinear ionic currents present in both pyramidal cells and the equivalent compartmental model, but can in fact be reduced to the above three rules. This reduction shows that the “divisive” computation performed by this mechanism is in fact a more complex operation, rather than a simple scaling factor applied equally across the firing range of the cell.

We suggest that due to the biophysical simplicity of the mechanism, and the ubiquity of its elementary components, many cell types have the potential to display this type of dendritic scaling of a DAP, leading to a division of neuronal output. Cortical cells in particular show the basic biophysical and anatomical requirements for displaying the deterministic gain control shown here. They have active dendritic sodium conductances (Stuart and Sakmann 1994), and spike timing is advanced by excitation (Reyes and Fetz 1993). Recent work has shown that balanced conductance fluctuations can attenuate backpropagating spikes in neocortical dendrites (Williams 2004). Further, the neocortex includes inhibitory interneurons exclusively contacting the soma or the dendrites.
(Markram et al. 2004). Such spatial segregation of synaptic inputs may allow the same computations we observe to be performed in the cortex.
4.1 Specific Introduction

A common problem faced by sensory systems is how to filter and parse complex stimuli within a single sensory modality to recognize and categorize behaviourally relevant signals from a complex sensory environment. Perhaps the simplest way of accomplishing this is to segregate the response of central neurons to primary afferent input into distinguishable subgroups of spike output. Pyramidal cells in the ELL of the weakly electric fish *A. leptorhynchus* display two modes of firing. One mode consists of a tonic firing regime characterized by regular spiking evoked by weak input currents. As injected current increases, the firing rate increases and eventually the cell progresses into a burst-firing mode (Lemon and Turner 2000; Turner et al. 1994). The bursting is characterized by an increasing firing rate that terminates in a high frequency doublet and post-burst pause before the cycle begins again (see Chapter 1; Figure 1-4).

Electric fish receive electrosensory signals with varying frequencies and degrees of spatial correlation (Chacron et al. 2003; Doiron et al. 2003a). In general, spatially “local” stimuli are related to prey or environmental objects; movement of the fish past these objects generates low frequency signals (MacIver et al. 2001). In comparison, spatially correlated “global” inputs are related to important conspecific signals for communication, including ‘chirps’ and beat frequencies generated by the difference in the frequency of EOD between different fish (usually > 20 Hz, E. Fortune, personal communication, (Heiligenberg 1973). These chirps often contain most of their power at
high frequencies (Zupanc et al. 2006). One effect of spatially correlated inputs is the recruitment of a direct inhibitory feedback pathway to the ELL (Doiron et al. 2003a; Doiron et al. 2004) that activates both GABA_A and GABA_B receptors on pyramidal cells (Berman and Maler 1998c). Of course, communication inputs do not preclude the existence of locally occurring prey-like signals, particularly since these fish and related species often forage in small groups (Tan et al. 2005, Eric Fortune, personal communication). Distinguishing between these different signals within a single sensory modality is a significant problem, analogous to the “cocktail party effect” discussed in human auditory processing (Cherry 1953; Haykin and Chen 2005), where specific frequencies within an input must be parsed.

As discussed in Chapter 1, pyramidal cells of the ELL are part of a direct closed loop feedback pathway with cells in the more rostral nP. Briefly, pyramidal cells send efferents to the nP bipolar cells, which in turn send inhibitory (GABAergic) fibres back to the ELL through the StF (Berman and Maler 1999; Maler and Mugnaini 1994) (Figure 1-3). This pathway is known to activate both GABA_A and GABA_B receptor-mediated IPSPs through Cl^- and K^+ conductances in pyramidal cells, respectively (Berman and Maler 1998c). The observed currents arise from synaptic contacts onto the somata and proximal apical dendrites of both E- and I-type ELL pyramidal cells (Maler and Mugnaini 1994) (Figure 4-1A). The fast GABA_A conductance has been shown to induce a gamma frequency oscillation in pyramidal cells due to a negative feedback that is recruited when the fish is exposed to electric fields with a spatial configuration similar to electrocommunication signals (Doiron et al. 2003a; Doiron et al. 2004; Lindner et al. 2005). The oscillation evoked by this input is on the order of 30-50 Hz, while the GABA_B
currents recorded in pyramidal cells have a duration of ~500 ms (Berman and Maler 1998c), far too slow to be involved in the genesis of this oscillation. The role of the slow GABA_B component of this feedback pathway has not previously been identified.

It has been shown that the burst mode of firing in pyramidal cells is caused by an interplay between somatic and dendritic spikes that involve a progressive shift in their temporal separation during repetitive discharge (Fernandez et al. 2005b). The work I present here now shows that the latency between somatic and dendritic spikes and the related burst discharge can be regulated by dendritic GABA_B inhibition from the descending feedback pathway. This regulation of the burst dynamics leads to an improved segregation of burst and isolated spike coding for low and high frequency input, respectively, a process that may regulate signal processing in vivo.

4.2 Results

4.2.1 GABA_B receptor activation alters burst dynamics

When stimulated with steps of intracellular current injection ELL pyramidal cells generally begin firing tonically and, with increasing current, shift into a burst firing mode (Figure 4-1B). Bursting is characterized by a progressive decrease in the ISI, terminating in a fast pair of spikes (‘doublet’ of 3-10 ms ISI) followed by a burst AHP (bAHP) (Lemon and Turner, 2000). This resets the burst cycle, causing a brief pause before the cycle begins again (Figure 4-1B1). The range of current injections between spike threshold and bursting is defined as the tonic range of firing (Figure 4-1D). In order to examine the effects of GABA_B receptor activation, we applied baclofen (100 μM) as a
selective GABA\textsubscript{B} agonist to the ELL PCL through focal pressure ejection from a small tipped pipette. Note that because GABAergic afferent axons from nP bipolar cells travel along the ventral StF, we could not use the StF as a barrier to segregate somatic and dendritic effects of ejected drugs, as in Chapter 3 and previous studies (Mehaffey et al. 2005; Noonan et al. 2003; Turner et al. 1994). Therefore, the possible locus for our observed baclofen effects include the activation of receptors at pyramidal cell somata and the most proximal regions of the apical and basilar dendrites (Maler and Mugnaini 1994).

Pressure ejections of baclofen in the PCL rapidly evoked a decrease if not a complete elimination of the tonic firing range of pyramidal cells. Rather, cells could repetitively fire spike doublets from the onset of firing, instead of exhibiting the normal range of tonic firing and then bursting (Figure 4-1B2). Figure 4-1D shows a representative FI plot displaying the full range of firing behaviours before and after baclofen ejections. Under control conditions, pyramidal cells fired faster and eventually began to burst as the driving current was increased. After baclofen ejection the tonic range of firing was abolished, and cells began to burst immediately upon crossing rheobase, effectively lowering the threshold for bursting (Figure 4-1B-D). Although not all cells showed a direct transition to bursting (n = 4 of 11), all pyramidal cells examined displayed a compression of the tonic firing regime, with an average 69 ± 5 % decrease in the tonic range of firing (p < 0.01; n = 11) (Figure 4-1C). Neurobiotin labelling showed that these effects occurred in both E- and I-type pyramidal cells (n = 4 I-cells, n = 3 E-cells).

Further, upon activation of inhibitory receptors, there was a corresponding increase in rheobase of 120 ± 40 pA (p < 0.05). Although this is consistent with the
Figure 4-1 A direct inhibitory feedback pathway can regulate bursting.

**A)** Schematic of direct feedback pathway to the ELL. Pyramidal cell axons project to nP bipolar cells, which in turn synapse on the pyramidal cell near the soma and along the most proximal apical dendritic region, activating both GABA\(_A\) and GABA\(_B\) receptor subtypes. **B)** Pyramidal cells fire tonically in response to current injection (**B1**, left, 0.2 nA). When the injected current surpasses a second threshold value the cell responds with patterned bursting (**B1**, right, 0.5 nA; arrows denote burst AHPs). Baclofen application to the soma/proximal dendritic region activates GABA\(_B\) receptors and the pyramidal cell now responds with spike doublets immediately after crossing spike threshold with no initial tonic firing (**B2**, 0.4 nA). **C)** Baclofen leads to a compression of the tonic range of firing, as indicated by a significant decrease in the range of current injections between spike threshold and bursting. **D)** Plots of the F-I relationship for tonic and burst discharge in a representative cell before and after focal pressure application of baclofen. Dashed lines indicate burst threshold (*black*), and the tonic firing range (*grey*) in control conditions.
predicted effects of inhibition, a compression of the tonic firing regime is distinct from the effects of inhibition in other cells. Most often, somatic inhibition leads to a subtractive change in rheobase, but does not alter the firing dynamics of the cell (e.g. Chapter 3, Chance et al. 2002; Mehaffey et al. 2005; Ulrich 2003). In this case, inhibition was able to qualitatively alter the firing behaviour of ELL pyramidal cells.

4.2.2 GABA<sub>B</sub> pathways in the ELL

The GABA<sub>B</sub> receptors affected by baclofen ejection could correspond to those normally activated by the direct feedback pathway through the StF that synapses on the soma and proximal apical dendrites of pyramidal cells, or by inputs from ovoid cells which synapse on the basal dendrites of I-type pyramidal cells (Bastian et al. 1993; Berman and Maler 1999; Maler and Mugnaini 1994). As the basal dendrite does not contribute to the burst mechanism in pyramidal cells (Turner et al. 1994), we can reduce the possible loci of GABA<sub>B</sub> inhibition that promotes burst discharge in I-type cells to either the soma or the proximal apical dendrite. E-type pyramidal cells exclusively receive GABA<sub>B</sub> mediated inhibition from nP bipolar cells (Berman and Maler 1998b). Since we observe compression of the tonic firing regime in both E- and I-type pyramidal cells, we suggest that the nP bipolar cell direct feedback pathway is the source of input to activate the GABA<sub>B</sub> currents mediating our results.
4.2.3 Stimulation of the StF can regulate burst threshold

In order to more rigorously test whether the StF is the source of the inhibition that regulates bursting, we compared the firing of pyramidal cells without, or immediately following activation of the StF fibre bundle (10x stimulation at 100 Hz). Stimulation of this pathway in the presence of DNQX generated an isolated slow, long-lasting IPSP of up to 500 ms (Figure 4-2A). The apparent absence of fast IPSPs is likely due to the cell being held near the reversal potential for Cl⁻ (~ -70 mV Berman and Maler 1998a) during stimulation. The slow IPSP has previously been shown to be GABA_B receptor-mediated and sensitive to common GABA_B antagonists (Berman and Maler 1998c). By pairing StF stimulation with a 200 ms depolarizing current pulse timed such that it overlapped the peak of the IPSP (after 50-100 ms delay following the end of the stimulus train), we were able to compare the firing frequency and burst thresholds of pyramidal cells with and without activation of the StF GABA_B pathway (Figure 4-2B). As found for baclofen ejections, stimulating the StF inhibitory pathway significantly compressed the tonic firing regime and increased the rate of burst firing (Figure 4-2C). The tonic firing range was compressed to 62 ± 8 % of the control range (p < 0.01, n = 5). Although no cells transitioned directly to bursting, this is consistent with the expected incomplete recruitment of GABA_B receptors during 100 Hz stimulation (Berman and Maler 1998c). Most importantly, these results suggest that direct StF stimulation of inhibitory inputs produces similar results to those evoked by focal pressure ejection of baclofen, confirming that the direct feedback pathway is sufficient to activate the necessary mechanisms to
Figure 4-2 Stimulation of the inhibitory component of the StF is capable of regulating bursting.

A) Stimulation of the nP bipolar cell feedback pathway in the StF (10 stimuli, 100 Hz) generates a slow IPSP. B) Pairing activation of the slow IPSP with a pulse of intracellular current (200 ms) allows the FI curve to be assessed during synaptic activation of GABA_B receptors. Arrows in the upper trace indicate StF stimuli, following which a clear hyperpolarization can be observed (dashed line). Lower trace shows the control record without synaptic stimulation, where no hyperpolarization is evident (dashed line). Stimulus artefacts have been truncated. C) Stimulation of the StF pathway regulates bursting, causing a compression of the tonic firing region. Dashed lines indicate the threshold for bursting, the point where the cell ceases tonic firing and begins to burst. Note that StF stimulation compresses the tonic firing range and lowers rheobase for burst firing.
regulate burst output via GABA$_B$ receptors in the somatic and proximal dendritic region of pyramidal cells.

4.2.4 *A two parameter bifurcation suggests a dendritic locus for GABA$_B$ mediated burst regulation.*

In order to analyze possible mechanisms for GABAergic compression of the tonic range of firing, we used a reduced two-compartment model of the ELL pyramidal cell that has been shown to accurately reproduce the dynamics underlying pyramidal cell bursts (Fernandez et al. 2005b). This model included the currents underlying spike discharge (Na$^+$, K$^+$) in both the somatic and apical dendritic compartments which were coupled through a resistance (Figure 4-3A; see Chapter 2 for full details). We began by performing a two parameter bifurcation analysis on the model using XPP-AUTO (Doedel 1981; Ermentrout 2002). Briefly, the two parameter bifurcation allows us to track either the saddle-node of fixed points (SNFP) or the saddle node of limit cycles (SNLC) bifurcation as a function of two parameters in the model (one of the two possible GABA conductances $g_{GABA_B}$, $g_{GABA_A}$ and the driving current $I$). These bifurcations correspond to the transition from quiescence to tonic spiking (SNFP), and from tonic spiking to bursting (SNLC), respectively (Fernandez et al. 2005b). We modeled GABA$_B$ receptor activation as a tonic K$^+$ conductance in either the dendritic compartment ($g_{dGABA_B}$) or somatic compartment ($g_{sGABA_B}$) and examined the effects of depolarizing the model through current injection (Figure 4-3A).
Figure 4-3 Two parameter bifurcation analysis of a reduced compartmental model using driving current and GABA conductances as test parameters.

A) Schematic diagram listing the currents involved in the two-compartment model. $R$ represents a coupling coefficient between somatic and dendritic compartments. B,C) Addition of GABA$_B$ conductance ($E_{rev} = -88.5$ mV) to the soma (B) raises tonic firing and burst threshold, but fails to compress the tonic range of firing. However, addition of this conductance to the dendrites (C) leads to a significant compression of the tonic range of firing and promotes an earlier shift to bursting. D,E) When a conductance reversing at -70 mV (e.g., a GABA$_A$-like conductance) is added to the soma, no compression of the tonic region is noticeable. A minor compression can be seen after addition of large amounts of the GABA$_A$ conductance to the dendritic compartment. F) An expanded view of the two parameter bifurcation for the GABA$_A$ conductance showing that large amounts of inhibition are able to compress the tonic firing regime, but require a larger conductance than that for the GABA$_B$ conductance. Note in particular, that the tonic firing regime collapses much more suddenly in the GABA$_A$ condition, as opposed to the graded compression observable in the GABA$_B$ condition (C).
When we performed a two parameter bifurcation analysis using GABA_B conductances placed somatically, we observed no change in the distance between the fold points associated with firing threshold and burst threshold - both points were right shifted by an equal amount such that no compression of the tonic firing range was observed (Figure 4-3B). After GABA_B conductances were placed in the dendrites we observed a compression of the tonic firing regime that increased with the level of dendritic GABA_B conductance (g_dGABA_B) (Figure 4-3C). This compression continued until the tonic regime was completely extinguished at g_dGABA_B \approx 0.4 \text{ mS/cm}^2. The transition to the stable oscillatory state (e.g. spiking) was still through a SNFP, so the cell still conformed to Type I excitability (e.g. the model remains capable of an infinitely slow approach to threshold, Rinzel and Ermentrout 1998). However, now each time that the cell crossed threshold, two spikes (the “doublet”) were created rather than one.

We note that our reduced model produced a large increase in firing threshold compared to our results from \textit{in vitro} recordings upon activation of dendritic GABA_B conductance. This discrepancy is due to the \(\kappa\) term in the model, which represents the relatively larger influence of the dendrite as compared to the soma. Although necessary to allow for the reduction of the entire dendrite to a single compartment, this leads to an overestimate of the effect of inhibitory dendritic conductances on the firing threshold of the cell. This reduction is valuable however, in that it allows us to perform the types of analysis discussed above, in particular the two-parameter bifurcation analysis, and has generated significant insights in both our system (Doiron et al. 2002; Fernandez et al. 2005b) and others (Pinsky and Rinzel 1994; Wang 1999). It has been shown previously
that in spatially extensive models and in real cells, dendritic inhibition in fact has less of an effect on spike threshold (Mehaffey et al. 2005), consistent with our in vitro results. Thus despite the overestimate of the rheobase shifts during dendritic inhibition, the model was able to reproduce the key salient feature (a compression of the tonic firing regime). Further, as we show below, the model also correctly predicts many features of dendritic inhibition on cell dynamics and coding properties.

Pyramidal cells also receive Cl⁻ mediated inhibition through GABA_A conductances from a wide variety of feedforward and feedback pathways that contact both the somata and dendrites (Berman and Maler 1998a; 1999). We therefore again carried out a two parameter bifurcation, but instead using an inhibitory conductance meant to replicate Cl⁻ mediated GABA_A inputs. When the GABA_A conductance was distributed somatically (\(g_{sGABA_A}\)), no compression of the tonic firing regime was obtained (Figure 4-3D), while a dendritic distribution (\(g_{dGABA_A}\)) led to only a slight compression of the tonic firing regime within an equivalent range (Figure 4-3E). Dendritic GABA_A was eventually able to abolish the tonic firing regime, but only at much larger conductances (Figure 4-3F). In order to examine the possibility of GABA_A modulation of pyramidal cell bursting we ejected muscimol (100 μM), a selective GABA_A agonist, into the PCL. As predicted by the model, muscimol failed to compress the firing regime of pyramidal cells significantly (9 ± 27 % of control, p > 0.05, n = 13; data not shown). Interestingly, the model predicts a more abrupt collapse of the tonic firing regime with GABA_A as compared to the more graded response observed with GABA_B inhibition. This may partially account for the lack of any observed effects during application of GABA_A
agonists. Our data and model therefore show that, within the physiological conductance range, hyperpolarization caused by GABA\textsubscript{A} inhibition at either proximal apical dendritic or somatic sites is not capable of compressing the tonic firing range.

4.2.5 Biophysical interpretation of burst regulation

The effects of GABA\textsubscript{B} activation on actual spike output is summarized in Figure 4-4. As documented in Figure 4-4B,C, adding a somatic GABA\textsubscript{B}-like inhibitory conductance ($g_{sGABA_B}$) to the model was ineffective at producing a shift to burst firing (Figure 4-4A,B). By comparison, dendritic GABA\textsubscript{B}-like inhibition ($g_{dGABA_B}$) replicated the decrease in tonic range of firing and the transition to a pure “doublet” mode of firing observed in our experimental results (Figure 4-4C). Our model thus suggests that the GABA\textsubscript{B} induced compression of the tonic firing range seen experimentally is due to inhibition of the apical dendrite. We therefore suggest that the compression of the tonic firing regime recorded \textit{in vitro} requires activation of dendritic GABA\textsubscript{B} receptors from the nP bipolar cell feedback pathway.

The change in the dynamics of pyramidal cell output invoked by dendritic GABA\textsubscript{B} conductances can be understood if we consider the normal dynamics of bursting in this system. Normally, spikes in ELL pyramidal cells are initiated at or near the soma and backpropagate along the dendrite. The dendritic spike is broader, and occurs with a delay relative to the somatic spike. The resulting voltage discrepancy between the somatic and dendritic compartments causes a dendro-somatic current flow (Figure 4-4E), giving rise to a DAP at the soma. In the normal dynamics,
Figure 4-4 A two compartment model suggests a mechanism for compression of the tonic firing range by GABA\textsubscript{B} receptor activation.

\textbf{A}) Somatic spike in the model under control conditions \((g_{GABA_B} = 0 \text{ mS/cm}^2)\). \textbf{B}) When GABA\textsubscript{B} currents are added to the soma \((g_{sGABA_B} = 0.4 \text{ mS/cm}^2)\) the spiking is unaffected. \textbf{C}) When identical currents are added to the dendritic compartment \((g_{dGABA_B} = 0.4 \text{ mS/cm}^2)\) the cell develops doublet spikes, replicating the results seen after baclofen application to pyramidal cells \textit{in vitro}. \textbf{D}) These dynamics can be replicated in the model by applying direct hyperpolarizing current injection to the dendritic compartment, suggesting that dendritic hyperpolarization is sufficient to regulate bursting. \textbf{E}) Schematic diagram of a pyramidal cell and superimposed records of somatic and dendritic activity in the model before and after a change in firing properties. In the model either dendritic hyperpolarization or addition of the GABA\textsubscript{B} current (shown here) delays the dendritic spike (2) relative to the somatic spike (1). This shifts the dendrosomatic feedback underlying a DAP (3) further outside the somatic refractory period, allowing the generation of a second somatic spike that signifies the transition to burst output.
burst doublets result when repetitive firing promotes a gradual decrease in the rate of rise of the dendritic spike due to Na\(^+\) channel inactivation (Fernandez et al. 2005b). The progressive delay in the peak of the dendritic spike acts to delay dendro-somatic current flow sufficiently such that the DAP falls outside of the somatic refractory period, causing a second somatic spike (Figure 4-4E). The ISI of the somatic spike doublet, however, falls within the refractory period of apical dendrites, resulting in the failure of backpropagation which terminates a burst.

The effects of dendritic GABA\(_B\) receptor activation could result through membrane hyperpolarization or the underlying conductance change. However, the model suggests that a shift to burst output does not require any change in membrane conductance, as the transition could be produced by hyperpolarizing dendritic current injection (Figure 4-4D). In the model, the effects of dendritic hyperpolarization were distinct in that membrane potential shifts lower than -75 mV augmented the transition to bursting by imposing an initial delay in the onset of the dendritic spike rather than slowing the rate of rise of the dendritic spike. As a result, the dendritic depolarization extended beyond the somatic refractory period to force a second spike, signifying the transition to burst firing (Figure 4-4E). In comparison, Cl\(^-\) mediated GABA\(_A\) conductances in the model with a reversal potential more depolarized than -75 mV do not hyperpolarize the dendrite sufficiently to delay the dendritic spike or invoke a graded compression of the tonic firing regime. The model thus provides a directly testable hypothesis for how the pyramidal cell may change its behaviour following activation of GABA conductances. Briefly, hyperpolarizing dendritic inhibition through GABA\(_B\) receptor activation should delay the dendritic spike in a voltage-dependent manner.
4.2.6 Dendritic inhibition selectively delays the onset and peak latency of dendritic spikes

In order to test the above hypothesis, we carried out recordings from pyramidal cell proximal dendrites near or within the StF. In control conditions the dendrite had a mean resting potential of -64.5 ± 1.1 mV (n = 5). After application of baclofen, the dendrite hyperpolarized to -81.4 ± 1.8 mV (p < 0.05). This hyperpolarization occurred along with an increase in latency of 0.87 ± 0.14 ms (p < 0.05; n = 4) between the antidromic stimulus and the peak of the dendritic spike. However, the shift in dendritic spike latency was not accompanied by a decrease in the rate of rise of the evoked spike (2 ± 5.0 %; n = 4, p > 0.05). In comparison, under these circumstances the soma hyperpolarized by only 4.7 ± 1.5 mV (n = 11), consistent with the degree of somatic hyperpolarization observed during stimulation of the inhibitory StF feedback pathway (Berman and Maler 1998c). In addition, the somatic antidromic spike did not display a significant change in delay (0.02 ± 0.05 ms of control, n = 3, p > 0.05) or rate of rise during baclofen application (Figure 4-5A,B). This confirms that inhibition due to baclofen application is insufficient to delay the somatic spike, and yet has a substantial effect on dendritic spike latency.

To determine if these effects on dendritic spike latency were attributable to the baclofen-induced conductance change or membrane hyperpolarization per se, we varied the resting potential of dendritic membrane through direct current injections. Cells were held between -55 and -85 mV and spikes were generated antidromically to measure the
Figure 4-5 Hyperpolarization delays the onset and peak of dendritic spikes in pyramidal cells.

A) Superimposed representative recordings of antidromic dendritic or somatic spikes before and after pressure application of baclofen (100 µM) in the PCL. Baclofen induces a large membrane hyperpolarization at dendritic sites and a pronounced increase in the delay of dendritic spike onset and peak latency with no change in spike rate of rise. By comparison, baclofen has little effect on somatic membrane potential, spike latency or rate of rise. Inset shows superimposed control and test dendritic spikes aligned for comparison of spike rate of rise. Stimulus artefacts are truncated. B) Plot of average increase in latency to peak of dendritic or somatic spikes before and after baclofen application, showing that spike latency is selectively increased in the dendrite. C) Representative example from a proximal dendritic recording of antidromic backpropagating spikes evoked from different levels of membrane potential superimposed for comparison. With increasing amounts of hyperpolarization, the onset and peak of the dendritic spike is increasingly delayed without affecting the rate of rise or repolarization. Inset shows all records superimposed and aligned to spike peak. D) Plots of the increase in spike latency observed in all dendritic recordings at varying holding potentials (n = 11). The region designated by grey shading corresponds to the range of increases in dendritic spike latency previously observed during burst discharge (Fernandez et al., 2005a), indicating that the shifts in dendritic spike latency during
hyperpolarization are representative of those known to occur during repetitive activity.

E) Summary plot of dendritic spike data shown in (D) indicating that a significant increase in dendritic spike latency occurs at voltages below ~72 mV. Data in (E) was binned in 5 mV increments.
delay between the stimulus and evoked spike. Here we found that injecting hyperpolarizing current also increased the peak delay of the antidromic dendritic spike in a voltage-dependent fashion by up to \( \sim 1.5 \) ms. Notably, this hyperpolarization did not change the rate of rise of the dendritic spike (4 \( \pm \) 3 \% change between the least and most hyperpolarized recordings, (Figure 4-5C, \( p > 0.05 \)). Spike delay was not significantly increased for voltages ranging between -55 mV and -72 mV (\( p > 0.05 \), one-way ANOVA), but showed a significant increase in delay when the resting membrane voltage was held at potentials of -75 mV or lower (0.28 \( \pm \) 0.06 ms, \( p < 0.05 \), one-way ANOVA), and increased further as the voltage approached -85 mV (0.57 +/- 0.08 ms, \( p < 0.05 \), one-way ANOVA, Tukeys HSD) (Figure 4-5D,E). Importantly, the delays in dendritic spike latency measured for either baclofen application or the more hyperpolarized holding potentials were within the range of those previously measured during repeated high-frequency antidromic stimulation (100 Hz) (Fernandez et al., 2005a). The shifts in dendritic spike latency thus fall within the normal range of increased delays that occur during burst discharge (Figure 4-5D, shaded area, 0.2 to 0.7 ms increase in latency, data from Fernandez et al., 2005a). Sufficient dendritic hyperpolarization (below \( \sim -72 \) mV) is thus capable of generating the delays observed during the repetitive firing leading up to bursts, and is consistent with the greater activation of GABA\(_B\)-induced hyperpolarization of dendritic membrane.

Taken together, these results suggest that, as in the model, GABA\(_B\) activation in pyramidal cells leads to a hyperpolarization of the dendrite, which in turn selectively delays the peak latency of the dendritic spike. By increasing the somato-dendritic spike latency the backpropagating spike can immediately transition the cell to burst firing
through an increase in the somatic DAP.

4.2.7 Compression of the bursting regime alters spike train segregation

After analyzing the mechanism responsible for GABA_B regulation of pyramidal cell bursting, we proceeded to determine the possible effects on information transmission in the ELL. In response to time-varying stimuli, the burst mechanism produces a bimodal ISI distribution that distinguishes bursts and isolated spikes of spike trains both \textit{in vivo} and \textit{in vitro} (Gabbiani et al. 1996; Oswald et al. 2004). It has previously been shown that bursts preferentially code for low frequency (e.g. prey-like and environmental) components of the stimulus, while isolated spikes code for both low and high frequency signals related to electrocommunication (Oswald et al. 2004). Our finding of a dendritic GABA_B regulation of burst discharge could thus modulate the coding strategies of pyramidal cells.

To test for GABA_B regulation of coding, we placed the model into two conditions based on the response to constant current injections. This consists of a control condition ($g_{dGABA_B} = 0.0 \text{ mS/cm}^2$) and a bursts-only condition ($g_{dGABA_B} = 0.4 \text{ mS/cm}^2$). The model was held near threshold as in previous studies (Oswald et al. 2004) and driven with a single instance of a noisy stimulus. These conditions allowed us to examine the effects of increased bursting on the model’s ability to code time-varying inputs. In particular, these effects cannot be explained by the increase in rheobase due to inhibition, as the cells were maintained near threshold. Therefore the following results are not due to inhibition causing a failure to respond to weak stimulus components but are due to the change in
intrinsic dynamics after dendritic inhibition. As a stimulus, we chose frozen RAMs filtered to contain power between 0 and 60 Hz, and compared the stimulus-response coherence with or without the dendritic GABA_B conductance. Figure 6 plots the coherence between the spike train and the RAM, with the spike train parsed into bursts and isolated spike components. When inhibition was placed in the dendrites, inducing the bursts-only regime (g_{dGABA_B} = 0.4 mS/cm^2), we observed a small increase in the burst coherence with high frequencies (defined as 30-50 Hz, Figure 4-6A). This was paired with a large decrease in the coherence of isolated spikes and low frequency components of the stimulus (defined as 0-20 Hz, Figure 4-6B). In comparison, the coherence between isolated spikes and high frequency components of the stimulus was not decreased.

Thus the non-linear interaction of dendritic inhibition with the intrinsic dynamics of the two-compartment model is able to significantly affect the coding of inputs. Closer inspection of data records revealed that this occurs because the increased sensitivity of the burst mechanism in the presence of dendritic inhibition prevents small amplitude, low frequency inputs from generating isolated spikes, restricting their occurrence to the high frequency components of the stimulus. Thus, isolated spikes are preferentially caused by high frequency components of the stimulus, as the sharp decrease of excitatory input due to fast stimulus downstrokes is able to prevent burst discharge. This suggests that GABA_B input to the proximal apical dendrites can switch the cell to a mode where the coding of broad-band signals becomes almost completely segregated such that bursts code for low frequencies and isolated spikes code preferentially for high frequencies.

We tested the model’s prediction in ELL pyramidal cells in vitro by activating GABA_B receptors with pressure ejection of baclofen while driving the cells from near
Figure 4-6 Compression of the tonic firing region redistributes spike segregation in the model.

The model is driven with a frozen white noise current injection at the soma and the stimulus-response coherence is calculated for burst and tonic spikes. A) During dendritic inhibition and compression of the tonic range of firing, the stimulus-response coherence for bursts of spikes is slightly increased (grey trace) relative to control (black trace) and remains preferentially coherent with low frequency components of the stimulus. B) In comparison, coherence between the stimulus and the isolated spikes decreases in the low frequency regions (grey trace) relative to control (black trace).
threshold with an identical frozen RAM stimulus to that used in the model \((n = 7)\). As shown previously \((\text{Oswald et al. 2004})\), a pyramidal cell’s response to a RAM under control conditions reflects an overall broad-band coherence to the stimulus when the combined output of isolated and burst spikes are considered \((\text{Figure 4-7A})\). The specific responsiveness of isolated or burst spikes can then be parsed out for separate consideration as performed for the model. This analysis shows that the response of isolated spikes in pyramidal cells to the RAM stimulus encompasses a relatively broad band component with a small peak in power at \(~40\) Hz \((\text{Figure 4-7A})\). Burst spikes instead show a preferential coherence to lower frequency components of the stimulus with a peak power \(~10\) Hz \((\text{Figure 4-7A})\).

After baclofen application had compressed the tonic range of firing, we observed effects similar to those predicted by the model with dendritic inhibition \((\text{cf. Figure 4-7, Figure 4-7B})\). In comparison to control, baclofen application caused a small increase in burst coherence at high frequencies. However, the coherence between isolated spikes and low frequency inputs was more dramatically reduced \((\text{Figure 4-7B})\). We quantified this by comparing the mean coherence for 0-20 Hz \((\text{low frequency})\) and for 30-50 Hz \((\text{high frequency})\) before and after application of baclofen. The coherence between bursts and high frequency events showed a modest but significant increase \((15 \pm 8\% \text{ from control}, p < 0.05)\), while coherence between bursts and low frequency events did not significantly change \((0.5 \pm 2\%, p > 0.05)\). As predicted by the model, the coherence between isolated spikes and high frequency events did not change \((\text{Figure 4-7B}; 2 \pm 5\%, p > 0.05)\). Rather, the most significant change was in the coherence between isolated spikes and low frequency events, which decreased by \(47 \pm 6\%\) relative to control values \((\text{Figure 4-7;})\).
A

Control

- All Spikes
- Burst Spikes
- Isolated Spikes

Baclofen

- All Spikes
- Burst Spikes
- Isolated Spikes

Bursts

- Control
- Baclofen

Isolated Spikes

C

% Change in coherence

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<th>Burst: High Frequency</th>
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Figure 4-7 Experimental confirmation of model predictions.

A) Application of baclofen to pyramidal cell in vitro causes a shift in firing properties that matches that in the model - the coherence of bursts is slightly increased (green trace) and the low frequency component of the isolated spike coherence (red trace) is reduced. Note that the overall coherence for all spikes (black trace) is minimally affected. B) Control coherence (black trace) versus coherence observed when the tonic firing range is reduced by baclofen (red trace) for burst spikes (left panel) and isolated spikes (right panel) plotted for comparison. C) Percent changes in the mean coherence for high (30-50 Hz) and low (0-20 Hz) frequency stimulus components and bursts or isolated spikes.
Further, we calculated the burst fraction as the percentage of bursts relative to the total number of spikes. Baclofen induced a significant increase in the burst fraction from 22 ± 4% to 31 ± 7% of all events (p < 0.05). Thus, the increase in bursting caused a redistribution of the stimulus-response coherence between bursts and isolated spikes. This allowed a greater segregation of separate components of complex stimuli, as summarized in Figure 4-7C.

4.3 Specific Discussion

Distinguishing between different signals imposed simultaneously on a sensory modality, and representing them as distinct spike trains, is a problem requiring specialized neural systems. In the electric fish, AMs of the electric organ discharge signal are caused both by prey (or inanimate objects) and communication signals from conspecifics. The frequency ranges of AM modulations associated with these signals is very different however: prey causes low frequency AMs (<20 Hz) while electrocommunication signals can range up to >200 Hz. One mechanism shown to be used by ELL pyramidal cells is to segregate behaviourally relevant frequencies of input by partitioning the spike train into distinct patterns of bursts and isolated spikes (Oswald et al. 2004). Work both in vitro and in vivo has established that bursts code selectively for low frequencies while isolated spikes are broadband and are able to code for the entire frequency range (Doiron et al. 2007; Oswald et al. 2004; Oswald et al. 2007). The extensive work completed on the dynamics of spike output in pyramidal cells allowed us to test the specific role of GABA_B receptors activated by an inhibitory feedback pathway.
on spike firing and coding in pyramidal cells. The present study shows that the dendritic GABA_B receptor component activated by descending feedback can regulate a burst mechanism intrinsic to pyramidal cells by shifting the relative timing of somatic spikes and backpropagating spikes to enhance burst output. In doing so, it induces an improved segregation of the spike train output by allowing isolated spikes to code preferentially for the high frequency range of input signals. We propose that the resulting increased segregation of spike firing will improve the ability to detect both prey-like objects (bursts) and communication signals (isolated spikes) when these two signals occur simultaneously. Such segregation of spike trains into distinct components related to different elements of the sensory environment may also be considered as a form of figure-ground discrimination, which can also have a strong temporal component (Fahle 1993). Although most commonly studied in the visual system, the auditory system appears to be capable of regulating neural activity in a similar fashion (Fritz et al. 2007). Spike train segregation may then be helpful in separating relevant environmental cues from a complex background, including communication calls and beat frequencies arising from differences in the EOD frequency between nearby fish.

In the context of figure-background discrimination, the mechanism described here will allow specific environmental signals (e.g. prey) to be distinguished from a complex background, without necessarily suppressing neural activity that represents this background. It should be noted, however, that the shift in rheobase associated with the inhibition observed here may contribute to such a background suppression. As isolated spikes have been proposed to be sufficient for signal reconstruction, while bursts detect low frequency features, the loss of isolated spikes may decrease the ability to accurately
encode low frequency events. Bursts, however, have been shown to be capable of accurately encoding low frequency events through their ISIs (Doiron et al. 2007; Oswald et al. 2007). This coding may be improved by more reliable bursting in response to low frequency stimuli. Understanding the final impact of shifts in spike segregation will require a greater understanding of the decoding of pyramidal cell spike trains by target midbrain neurons.

4.3.1 GABA<sub>B</sub> feedback and environmental context

A schematic of our suggested model of the function of the GABA<sub>B</sub> feedback pathway is shown in Figure 4-8. When a conspecific is present (Figure 4-8A), the activation of ELL pyramidal cells by the sensory stimulus recruits the direct feedback pathway from nP bipolar cells (Figure 4-8B). This causes both a ~30-50 Hz GABA<sub>A</sub> mediated oscillation (Doiron et al. 2003a) and a more tonic inhibition by the slower GABA<sub>B</sub> receptors (Figure 4-8C). The longer lasting GABA<sub>B</sub> mediated hyperpolarization delays the onset of the pyramidal cell backpropagating dendritic spike (Figure 4-8D), and thereby compresses the tonic firing range. The longer latency dendritic spike increases a dendrosomatic current flow to increase bursting and allows a greater segregation of information transmission by the spike train. Therefore, as a result of the GABA<sub>B</sub> inhibitory feedback, isolated spikes now code preferentially for the high frequency component of the broad band input signal (Figure 4-8E).
Figure 4-8 Schematic of proposed mechanism to account for the shift in spike firing properties invoked by dendritic GABA_B receptor activation.

Spatially correlated conspecific signals (A) recruit the nP bipolar cell direct feedback pathway to ELL pyramidal cells (B). This leads to the activation of two synaptic currents: a GABA_A mediated signal shown to drive synchronous oscillations (Doiron et al. 2003a), and a more prolonged GABA_B signal in the proximal dendritic and somatic region (C). GABA_B receptor activation leads to a decrease in burst threshold by modulating dendritic spike latency (D). The decrease in burst threshold increases the segregation between burst and isolated spikes by increasing the specificity of isolated spikes for high frequency signal components, so that they code entirely for conspecific signals (indicated by coherence plots in E).
4.3.2 Bursting in pyramidal cells

The burst dynamics of ELL pyramidal cells are well understood (Fernandez et al. 2005b; Lemon and Turner 2000; Turner et al. 1994), have been extensively modeled (Doiron et al. 2002; Fernandez et al. 2005b; Laing et al. 2003; Laing and Longtin 2002), and analyzed with respect to their role in sensory processing (Doiron et al. 2007; Oswald et al. 2004; Oswald et al. 2007). This burst firing depends on an increasing delay that develops between the somatic spike and backpropagating dendritic spike during repetitive discharge (Fernandez et al., 2005a). The key variable for this delay is a cumulative dendritic Na⁺ channel inactivation, as reflected in a slowing of the rate of rise of the dendritic spike during a burst. The resulting increase in temporal separation of somatic and dendritic spikes accentuates a dendrosomatic current flow that can influence the spike generating mechanism (Fernandez et al., 2005a). Normally this process builds gradually during repetitive spike firing until the dendritic spike occurs sufficiently outside the somatic refractory period as to generate a somatic spike ‘doublet’ that terminates the burst.

An analysis of our reduced model of burst dynamics and direct recordings from pyramidal cells suggests that GABAᵦ inhibition is capable of delaying the dendritic spike sufficiently to bypass the gradual buildup to bursting and immediately promote spike doublets. This inhibition-induced delay of the dendritic spike required only strong hyperpolarization from synaptic currents (with $E_{rev} < -75$ mV) or from hyperpolarizing current injection in both the model and pyramidal cells. Moreover, it involved a shift in
the onset latency of the dendritic spike with no effect on either spike rate of rise or amplitude. Dendritic GABA_B inhibition then represents a second means by which the temporal relationship between somatic and dendritic spikes can be altered.

Bipolar cells of the nP that project in the direct feedback pathway are predicted to drive the GABA_B inhibition of pyramidal cell dendrites under natural conditions. This pathway is important in that it is preferentially recruited during communication-like stimuli (Doiron et al. 2003a) to activate both GABA_A and GABA_B receptors on pyramidal cells (Berman and Maler 1998c). Despite the inhibitory nature of this pathway, its activation in vivo leads to an increase in the number of brief ISIs (5-8 ms) that reflect intrinsic bursts in pyramidal cells (Doiron et al. 2003, Supplemental Materials), as well as inducing network mediated oscillatory activity (Doiron et al. 2003a). It was suggested in these earlier in vivo studies that bipolar cell feedback may be responsible for increasing pyramidal cell bursts through an unknown mechanism. We find that the increase in burst activity can be accounted for when proximal dendritic GABA_B inhibition compresses the tonic firing range. This is observable in the response to RAMs, where the burst fraction is significantly increased during manipulations that compress the tonic firing regime. This regulation of burst dynamics is due to a shift in dendritic spike latency and causes a concomitant shift in the role of isolated spikes in encoding high frequency inputs. This effect may be translated into an improved ability to detect both prey-like and communication signals.

4.3.3 Bursting and network oscillations

We note that this process is distinct from an alternative mechanism identified in
pyramidal cells in which behaviourally relevant inputs induce a network mediated gamma oscillation that is only evoked by spatially global input arising from electrocommunication signals. The latter mechanism was shown to arise from activation of a delayed inhibitory feedback pathway from the nP that activates GABA_A receptors on pyramidal cells (Doiron et al. 2003a; Doiron et al. 2004). Further studies of the feedback-induced gamma oscillation have shown that it requires fairly high frequency input (>40 Hz) that can only be due to communication signals (Doiron, Bastian and Maler, personal communication). We focus on the interaction between this network mediated oscillation and the intrinsic bursting in the following chapter (Chapter 5).

Work in ELL pyramidal cells thus identifies distinct roles for GABA receptor mediated inhibition activated by a descending feedback pathway in segregating spike trains for the purpose of sensory coding. It also identifies a particular role for dendritic inhibition in this process. We expect that this strategy will be active in other cells, in that a decrease in the relative delay between somatic and dendritic spikes with depolarizing dendritic current has been reported (Stuart and Hausser 1994; Stuart and Sakmann 1994). GABA_B inhibition was also recently shown to increase the delay between the somatic spike and backpropagating spike in hippocampus (Leung and Peloquin 2006), although potential changes in the cell’s dynamics were not examined. These similarities would suggest that our results will have wide application in signifying that the mean voltage of dendritic compartments can regulate the timing of backpropagating dendritic spikes, with corresponding effects on soma-dendritic interactions.
4.3.4 Functions of somatic and dendritic GABA receptor activation

The effects of inhibition on ELL pyramidal cell spike output are multifaceted. For instance, GABA_A inhibition can cause subtractive or divisive effects on cell output depending on the location of the synapses (Mehaffey et al. 2005). Further, in the intact animal subtractive somatic inhibition is capable of creating oscillatory activity due to feedback delays (Doiron et al. 2003a). GABA_B mediated inhibition targeting the basal dendrite acts as a low pass filter to support coding for the envelope of a narrow band high frequency signal (Middleton et al. 2006). We now show with the present results that the proximal apical dendritic GABA_B receptors (but not GABA_A) can selectively regulate burst dynamics in relation to activity in a specific feedback pathway. It is thus important to consider not only the type and time scale of inhibition but also the site of synaptic termination to understand the effects of inhibition on both total synaptic input and the cell’s intrinsic firing dynamics.

Differential impacts of somatic and dendritic inhibition on the electrophysiological behaviour of neurons have been reported previously (Mehaffey et al. 2005; Vu and Krasne 1992) and may allow different sets of inhibitory interneurons to differentially regulate patterns of spike generation. The nervous system contains a wide variety of interneurons, whose roles are only recently beginning to be elucidated (Soltesz 2006). We previously described how a weaker GABA_A mediated dendritic inhibition acts to reduce the gain of the FI relationship in a divisive manner by altering dendritic spike shape (Mehaffey et al. 2005). No reduction in gain upon GABA_B receptor activation was observed here or in previous studies (Berman and Maler 1998c). The effects of GABA_A
receptor activation on dendritic spikes were different, however, in only reducing the amplitude of the late phase of the dendritic spike, while GABA_B conductances increased the relative latency between the dendritic and somatic spikes. The apparent lack of effect by GABA_B receptors on the late phase of the dendritic spike may simply reflect the more active components of spike discharge in proximal dendritic regions. The earlier study of Mehaffey et al. (2005) focused on more distal dendritic GABA_A (~100 μm) conductances that regulated the shape of the backpropagating spike. Proximal GABA_B inhibition may be unable to sufficiently regulate the narrower dendritic spike waveform inherent to more proximal regions of the dendrite, preventing the decrease in gain seen with GABA_A agonists. The localization of the GABA_B receptor appears to be primarily dendritic, as shown by the increased hyperpolarization of dendritic membrane voltage by baclofen. The somatic membrane hyperpolarization may be due to somatic GABA_B receptors, or to the dendritic inhibition. Somatic GABA_B receptors would be hypothesized to have a primarily subtractive effect, filtering out small amplitude signals, but not changing the firing dynamics of the cell significantly.

4.3.5 Possible interactions with other components of ELL architecture

One further network effect predicted by the anatomy of the ELL (Maler and Mugnaini 1994) involves the inhibition of the VML cells responsible for many of the projections that activate GABA_A receptors in the proximal apical dendrites (Berman and Maler 1998a). Previous studies suggested that the nP bipolar cell provided input to VML cell somata (Maler and Mugnaini, 1994). Since VML cell mediated inhibition reduces the
gain of pyramidal cells (Mehaffey et al. 2005), inhibition of this interneuron should, in turn, increase the gain of pyramidal cells.

We show here that feedback-evoked synaptic currents qualitatively alter the frequency-dependent intrinsic dynamics of ELL pyramidal cells. This regulation of intrinsic pyramidal cell dynamics is predicted to occur in the specific context of communication-like signals, i.e. when these fish are foraging in groups. It has recently been shown in a related electric fish species that behaviourally induced gamma range oscillations actually improve the ability of the animal to detect prey, possibly by inducing short term depression in target cells in the TS (Ramcharitar et al. 2006). We therefore propose that, when these fish forage in groups, the bipolar cell mediated feedback inhibition has three effects that act synergistically to improve the ability of the fish to detect moving prey (low frequency) while also receiving signals from conspecifics (including high frequencies).

Firstly, the bipolar cell feedback inhibition (GABA_A component) evoked by the communication signals will induce a gamma oscillation that will improve the directional selectivity of TS neurons for moving prey. Secondly, the same inhibitory pathway will, via its GABA_B component, switch the isolated spikes into coding selectively for high frequency communication signals; this segregation will presumably improve the ability of TS neurons to disambiguate prey and communication signals. Thirdly, the bipolar cell inhibition of VML cells will increase the gain of pyramidal cells, again improving their ability to code for moving prey. Thus the complex interaction of network, synaptic and intrinsic dynamics might be required to generate a simple final result- an enhanced ability for the fish’s detection of prey while foraging in groups.
Chapter Five: Feedback Modulation of Firing Dynamics Restores Feature Detection in Electrosensory Processing

5.1 Specific Introduction

One vital computation performed by the nervous system of any animal is the parsing of complex stimuli from the outside world in order to recognize behaviourally relevant signals. Examples include the auditory stream segregation problem commonly referred to as the “cocktail party effect”, or the problem of figure-background segregation in the visual system. One simple way of accomplishing this is to segregate the response of central neurons to primary afferent input into distinguishable subgroups or patterns of spike output.

Electrosensory neurons require the ability to code distinct frequency ranges of afferent input, as they receive electrosensory inputs with varying frequencies and degrees of spatial correlation (Chacron et al. 2003; Doiron et al. 2003a). In general, spatially correlated (“global”) inputs are related to conspecific signals for communication, which contain most of their power at high frequencies (but see Chapter 1) (Zupanc et al. 2006). In comparison, spatially distinct (“local”) signals are related to prey or environmental objects, and these contain power at lower frequencies (MacIver et al. 2001). As discussed in Chapter 4, one important effect of spatially correlated, high frequency inputs involves the recruitment of an inhibitory feedback pathway from bipolar cells of the nP to the ELL (Bastian et al. 2004; Berman and Maler 1999; Doiron et al. 2003a). Activity of nP bipolar cells gives rise to activation of descending pathways that activate both GABA_A and GABA_B receptors on pyramidal cells (Berman and Maler 1998c; Mehaffey
et al. 2007).

We have shown previously that the burst mode of firing in ELL pyramidal cells results from an interplay between somatic and dendritic spikes due to a progressive shift in their temporal separation during repetitive discharge (Fernandez et al. 2005b), and that this delay can be directly modulated by dendritic GABAB receptor mediated inhibition (Mehaffey et al. 2007) (Chapter 4). Specifically, we have shown that the latency between somatic and dendritic spikes and the related burst discharge in pyramidal cells can be regulated by dendritic GABAB receptor activation that arises from nP bipolar cell input. An important aspect of this regulation of pyramidal cell burst dynamics is an improved segregation of burst and isolated spikes coding for low and high frequency input, respectively. Note that nP bipolar cells are recruited during situations where conspecifics are likely to be present (Doiron et al. 2003a). nP bipolar cell feedback may therefore improve the parsing of the frequency content of broadband input into distinct patterns of spikes in pyramidal cells relevant to distinguishing environmental and communication signals (Mehaffey et al. 2007). The experiments and modeling previously used to investigate the actions of GABA_B receptors were all performed \textit{in vitro} using tonic GABA_B receptor activation due to the restrictions inherent to pharmacological manipulations in the slice, and to allow dynamical analysis. However, GABA_B mediated feedback \textit{in vivo} should be recruited concurrent with GABA_A mediated inhibition that also results from the nP bipolar cell feedback.

Work \textit{in vivo} has shown that GABA_A receptor activation by nP feedback is important to establishing a gamma frequency oscillation in pyramidal cell discharge during presentation of the global, correlated inputs characteristic of
electrocommunication signals (Doiron et al. 2003a). This oscillation is created by the network architecture, where pyramidal cells excite nP bipolar cells, which in turn inhibit pyramidal cells with a delay due to the conduction time (measured at ~ 15 ms). This form of closed loop inhibitory network easily gives rise to oscillatory activity when delays are sufficiently long (Doiron et al. 2003a; Doiron et al. 2004; Lindner et al. 2005), and has been suggested to underlie ELL pyramidal cell oscillations observed in vivo. One means of establishing this was to use an elegant functional dissection of the nP inhibitory feedback pathway in vivo through focal ejections of lidocaine in the lateral lemniscus rostral to the ELL (Doiron et al. 2003a). The authors further used LIF or reduced models to identify GABA_A receptor activation by nP inputs as a critical component of pyramidal cell gamma frequency oscillations. However, the role of GABA_B receptor activation in this network was not considered, and the reduced models used in Doiron et al. (2003) incorporated a different scheme of ion channel kinetics underlying intrinsic burst activity. Given the important role we have shown for GABA_B receptor activation in regulating burst dynamics of pyramidal cells, it is important to assess whether periodic activation of GABA_B receptors by nP feedback plays a role in (i) influencing gamma-frequency oscillations, or (ii) modifying signal processing capabilities of pyramidal cells in the presence of correlated receptor activation.

We now expand upon this initial work to test the role of GABA_B receptor activation in pyramidal cell gamma oscillations and signal processing capabilities by modeling a small network consisting of many individual two-compartment model pyramidal cells coupled through diffuse feedback that incorporates the GABA_A and/or GABA_B component of input from the nP. Full details of the model and related
methodology are contained in Chapter 2, and diagrammed in Figure 2-2B.

5.2 Results

The studies by Doiron et al (2003) testing the mechanism underlying gamma frequency oscillations used LIF models to establish the basic phenomena. They also used a reduced compartmental model (see their Supplemental Materials), although this was based on a different ion channel kinetic scheme to generate burst output (Doiron et al. 2002). We began by replicating the results of Doiron et al. (2003) using our reduced compartmental model of ELL pyramidal cell dynamics (Fernandez et al. 2005b). Note that this model represents the most recent and most accurate representation of conditional backpropagation based on dendritic Na\(^+\) channel inactivation, and one that is able to replicate the burst regulation we have observed by GABA\(_B\) mediated conductances (Mehaffey et al. 2007), Chapter 4). However, we initially needed to confirm that our network activity was consistent with that previously used to study the nP-ELL feedback loop. Briefly, this network was constructed out of 100 two-compartment pyramidal cell models coupled through a feedback kernel which transformed their spiking events into delayed inhibition (see Methods, Chapter 2). As done previously (Doiron et al. 2003), this network was driven with low-pass filtered Gaussian white noise (0-60 Hz), or with an independent Ornstein-Uhlenbeck (OU) noise process to provide independent, uncorrelated inputs. This gives us a time-varying stimulus and allows us to control the degree of input correlations to the network. A further, additive noise source was included
via another OU process with a faster correlation time constant to simulate spontaneous noise in the system (see Methods, Chapter 2).

The resulting networks and associated raster plots of spike times in model pyramidal cells are shown in Figure 5-1A-C. Briefly, we found that uncorrelated inputs are incapable of recruiting the network mediated oscillation whether the network uses GABA_A mediated feedback or combined GABA_A/GABA_B mediated feedback (Figure 5-1A-B). In contrast, correlated inputs are able to drive the network synchronously in the presence or absence of GABA_B-mediated inhibition (Figure 5-1C,D). This shows that the inclusion of GABA_B currents in the network model does not affect the basic results described previously (Doiron et al. 2003). Moreover, it establishes that synchronous recruitment of the population of model pyramidal cells requires correlated inputs (e.g. globally coherent sensory inputs). By comparison, even with the GABA_B mediated feedback currents included, inhibition is not strong enough to synchronize firing activity in the network when it is driven with uncorrelated inputs.

5.2.1 \textit{GABA}_A \textit{but not GABA}_B receptor activation mediates a network-mediated delay induced oscillation. \vspace{0.1cm}

When the network is driven with uncorrelated (local) inputs, the autocorrelation A(\tau) of a single model cell shows a single peak, which then decays to a flat autocorrelation (Figure 5-2A). When the cells of the network are driven with a shared input, an oscillation appears in the autocorrelation due to the coordinated recruitment of feedback inhibition (Doiron et al. 2003a). I repeated this test using our more
Figure 5-1 Network schematics and associated raster plots of pyramidal cell spike discharge.

Shown is a network model comprised of 100 reduced model pyramidal cells with a burst mechanism based on that delineated in Fernandez et al. (2005) and activated by either local (uncorrelated) or global (correlated) excitatory input from primary afferents, schematized in the form of a short section of the RAM stimulus. GABA_A connectivity from the nP is included in all cases. A feedback delay of 15 ms is implemented to simulate the delay induced by the conduction time between the nP and ELL. A) When all cells are driven with local, uncorrelated inputs and receive only the GABA_A component of nP feedback, no structure is apparent in the raster plots of pyramidal cell discharge in the network model. B) Including the GABA_B component of the feedback does not cause patterned activity of pyramidal cell firing in response to uncorrelated input. In comparison, cells driven with global, correlated inputs and nP feedback comprised of either GABA_A alone (C) or GABA_A and GABA_B receptor activation (D) show a structured activity in the population due to both the coordinated recruitment of the feedback pathway as well as the shared input.
biophysiologically realistic reduced compartmental model. When cells are driven with uncorrelated inputs, the autocorrelation peaks once and then decays to a steady state (Figure 5-2A). In comparison, when cells are driven with correlated inputs, delayed feedback from the nP is able to generate an oscillation, which is clearly observable in an autocorrelation of the pyramidal cell spike train (Figure 5-2B). Including GABA\textsubscript{B} receptor activation in the feedback causes a leftward shift in the peak of the autocorrelation (Figure 5-2C), but neither prevents nor encourages the creation of an oscillation. We then calculated the oscillation index (OI) by calculating the difference between the nadir after the first peak and the second peak of the oscillation. When the network is driven by uncorrelated inputs, no oscillation is present as there is no second peak observable, and the resulting OI is nonexistent (Figure 5-2A). In contrast, when cells in the network are driven with shared and correlated inputs, the resulting OI is approximately 7, indicating an oscillation is present. Including GABA\textsubscript{B} feedback slightly reduces the OI to near 6.5, but leaves the oscillation qualitatively intact. This shows that GABA\textsubscript{B} receptor activation does not appear to strongly modulate the network dynamics underlying the fast oscillation related to feedback delays in pyramidal cell firing as a whole.

\textit{5.2.2 A slow time constant prevents GABA\textsubscript{B} mediated conductances from driving oscillatory behaviour}

As GABA\textsubscript{B} mediated hyperpolarizations have a slow time constant, its minimal effects on the fast network oscillation can be considered consistent with an activation
Figure 5-2 Correlated inputs cause a network oscillation.

A) Pyramidal cell spike train autocorrelation for uncorrelated inputs when model cells receive both GABA_A and GABA_B nP feedback. Note that there are no significant correlations past the initial peak. B) When the network is presented with correlated inputs and only GABA_A feedback, an oscillation is apparent in the spike train autocorrelation. C) Including GABA_B receptor activation in the feedback pathway causes a shift in the peak of the autocorrelation to a shorter ISI, revealing correlations in the ISI structure that are consistent with increased bursting.
pattern outside of the time frame necessary to underlie this response. However, some remnants of the original oscillation frequency driving GABA\textsubscript{B} activity may still be apparent in the resulting conductance, even after being filtered through the slow synaptic dynamics. In order to test the degree to which GABA\textsubscript{B} feedback contributes to network-mediated oscillations, we examined the synaptic conductances contributing to the correlated input condition.

We thus calculated autocorrelations of the synaptic currents generated in the model under conditions of correlated input and both GABA\textsubscript{A} and GABA\textsubscript{B} components of nP feedback. This revealed that GABA\textsubscript{A} receptor activation shows a significant oscillation that can contribute to the network oscillation (Figure 5-3A). In contrast, GABA\textsubscript{B} receptor activation showed no significant oscillation (Figure 5-3B), but instead maintained a measurable, but steady value near 0.2 $\mu$S/cm\textsuperscript{2}. We therefore conclude that in our model network, GABA\textsubscript{A} feedback from the nP drives the network oscillation, a finding consistent with a previous study where cells coupled exclusively through GABA\textsubscript{A} receptor activation were sufficient to create the oscillation (Doiron et al. 2003a). Thus, the resulting activation of GABA\textsubscript{B} receptors is relatively tonic, similar to the methodology used in our earlier studies (Mehaffey et al. 2007). However, the question remains as to what contribution the GABA\textsubscript{B} feedback component may make to sensory coding during these oscillations, and how the shifts in burst dynamics resulting from relatively tonic GABA\textsubscript{B} activation interact with the fast network oscillations due to
Figure 5-3 GABA_\text{A} \text{ receptor mediated feedback is exclusively responsible for the network mediated oscillation.}

A) Autocorrelation of the synaptic GABA_\text{A} current under conditions of correlated input and both GABA_\text{A} and GABA_\text{B} inhibitory nP feedback pathways. A clear oscillation in the autocorrelation of the resulting GABA_\text{A} mediated synaptic current is observable. In contrast, the autocorrelation of the GABA_\text{B} synaptic conductance is flat, suggesting no contribution of GABA_\text{B} mediated inhibitory feedback to the oscillations observed in pyramidal cell spike trains during presentation of correlated input (B). Thus, the periodic drive which recruits GABA_\text{B} activity is filtered by the slow time constant of GABA_\text{B} synaptic dynamics, producing a largely tonic conductance.
5.2.3 *GABA*<sub>B</sub> mediated feedback promotes pyramidal cell bursting

We first examined whether *GABA*<sub>B</sub> mediated feedback was able to shift the pyramidal cell ISI histogram to include shorter ISIs, as predicted by its ability to regulate dendritic backpropagation and promote bursting in these cells (Mehaffey et al. 2007). When presented with uncorrelated inputs the ISI histograms are unimodal with an early peak, reflecting the lack of network mediated oscillatory activity (Figure 5-4A). Adding *GABA*<sub>B</sub> feedback in the model has no significant effect on ISI histograms, as in the uncorrelated condition, the *GABA*<sub>B</sub> conductance is not recruited (0 μS/cm<sup>2</sup>), although small differences between the two histograms are revealed upon subtraction (Figure 5-4A). These differences are due to different instances of the independent driving currents, as well as to the added noise, but do not show any concerted shift in the ISI histogram. In contrast, during presentation of correlated primary afferent input, ISI histograms of pyramidal-cell firing exhibit a distinct bimodality in association with the network mediated oscillation (Figure 5-4B). Including *GABA*<sub>B</sub> feedback in the network driven by correlated inputs then revealed a concerted shift in the ISI histogram to include a greater proportion of short ISIs (Figure 5-4B).

This increase in short ISIs is specifically in the range of those reflecting burst events, as verified by close inspection of sequential ISIs that revealed the stereotypic sequence indicative of conditional backpropagation and burst discharge (not shown).
Figure 5-4 GABA\textsubscript{B} mediated feedback causes a shift in the peaks of pyramidal cell ISI histograms.

Shown are schematic diagrams of the network model depicting the two conditions of uncorrelated (A) or correlated (B) primary afferent input and pyramidal cell ISI histograms in the presence or absence of GABA\textsubscript{B} mediated nP feedback. When the network is presented with uncorrelated inputs (A), the addition of GABA\textsubscript{B} to the feedback pathway does not cause an increase in the number of short ISIs. When the network is presented with correlated inputs (B), a multimodal ISI histogram is evoked that reflects the network oscillatory pattern. Adding GABA\textsubscript{B} feedback causes a shift in the ISI histogram towards shorter ISIs (arrowhead in the plot of differences between ISI histograms), indicative of burst firing. The range of ISI’s associated with bursting are indicated by a grey bar.
Therefore, as in previous studies, inclusion of the GABA_B feedback pathway causes a shift in the cell’s dynamics toward burst discharge (Mehaffey et al. 2007). Moreover, this effect is not precluded by the network oscillation. Previous work on this feedback pathway had revealed a significantly greater number of bursts during *in vivo* recordings when the combined inhibitory pathways are intact (Doiron et al. 2003a) (Supplemental Materials). We thus compared our observations to data recorded in vivo (Figure 5-5A). A plot of the difference ISI revealed an early peak of short ISIs associated with the loss of the component of inhibitory feedback *in vivo* upon blockade of the feedback pathway with focal injection of lidocaine (Doiron et al. 2003). We can then compare this result to the difference in the ISI histograms under equivalent conditions in the model. We therefore take the difference in ISI histograms between a cell given a frozen noise input in the uncorrelated input condition and a cell in which the entire network received a correlated frozen noise stimulus. This was done in a model network which either included, or did not include, a GABA_B mediated component to nP feedback. Without GABA_B mediated feedback no increase in the number of short duration burst ISIs was observed (Figure 5-5B), while including GABA_B feedback led to a leftward shift in the ISI histogram (Figure 5-5C). Note that this is a subtraction between an intact feedback pathway and a complete block of the feedback pathway, unlike Figure 5-4 where only the GABA_B component of the model is removed. This showed that the GABA_B mediated portion of feedback is required in our model to replicate the shifts in the ISI histogram observed in previous *in vivo* recordings (Doiron et al. 2003a) (Supplemental Materials).
Figure 5-5 The effects of removing the nP bipolar feedback pathway \textit{in vivo} can be replicated by the network model.

A-C) A comparison of plots of the difference between ISI histograms of pyramidal cell discharge recorded \textit{in vivo} or in the network model with and without the complete nP feedback inhibition (accomplished \textit{in vivo} through focal pressure ejection of lidocaine) (Doiron et al. 2003a). ISI difference plots of \textit{in vivo} data (A) show that when the nP bipolar cell feedback pathway is intact, there is a higher likelihood that pyramidal cells will fire short ISIs associated with brief bursts of spikes (arrow, gray shading). B) In the network model the nP GABA\textsubscript{A} feedback pathway alone fails to cause a shift in the ISI histogram of pyramidal cell spike output. Including the GABA\textsubscript{B} component of the feedback pathway, however, is able to replicate the shift in the ISI histogram observed \textit{in vivo} when inhibition is intact (arrow) (C). \textit{In vivo} data courtesy of Dr. J. Bastian, University of Oklahoma.
5.2.4 Gamma oscillation prevents accurate linear encoding of stimuli.

The effects of interactions between the network mediated oscillation and intrinsic burst dynamics of pyramidal cells on sensory coding remains unclear. In order to examine the possible regulation of sensory coding, we calculated the coding fraction ($\gamma$) of individual model cells within the network in response to either correlated or uncorrelated inputs, as well as with and without nP bipolar cell GABA\(_B\) mediated feedback. The coding fraction represents the proportion of the original stimulus that can be reconstructed from the optimal linear reconstruction kernel, e.g. the ability of the cell to code linearly for the stimulus (Gabbiani and Metzner 1999; Gabbiani et al. 1996; Metzner et al. 1998)

Under control conditions, $\gamma$ in the model was 0.3 in response to uncorrelated primary afferent input (Figure 5-6A). We observed a decrease in the coding fraction when the network was driven with correlated inputs ($\gamma = 0.255$), concomitant with recruitment of the GABA\(_A\) mediated feedback pathway. This is due to the delayed inhibition underlying the oscillation, which creates an interfering signal and reduces the ability of the individual cells to code for the original stimulus. As inhibition can prevent spiking from occurring during a stimulus-related depolarization, there will necessarily be small regions occurring at $\sim 30$ Hz which are no longer encoded by the spike train in the presence of an independent signal. In fact, this decrease in the coding fraction is similar to the observation in vivo that the oscillatory activity reduced the accuracy of the linear
Figure 5-6 Including GABA_B regulated burst and isolated spike stimulus-response coherence.

Plots of the coding fraction and burst fraction of pyramidal cell output in the network model to correlated or uncorrelated primary afferent input with or without nP GABA_B mediated feedback. A) In the uncorrelated (e.g. non-oscillatory) state of the network, the coding fraction is highest. The GABA_A mediated network oscillation has a deleterious effect on the coding fraction, and including the GABA_B component of the feedback causes a further decline in coding fraction. B) In comparison, the burst fraction is reduced due to the GABA_A mediated network oscillation. The burst fraction can however, be restored by including the GABA_B feedback, whose activity can modulate the intrinsic burst dynamics of the individual cells (Mehaffey et al. 2007).
estimate of the original stimulus (Doiron et al. 2003a). We found that inclusion of GABA_B mediated feedback in the model failed to improve coding accuracy, but instead caused a further decrease in the coding fraction ($\gamma = 0.213$) (Figure 5-6A). However, by examining the effects on burst fraction (e.g. the fraction of all events classified as bursts, see Chapter 2), we found that the GABA_B mediated increase in short ISIs (e.g. burst events) was able to correct for a decrease in the burst fraction due to GABA_A receptor activation during oscillations (Figure 5-6B). Thus, although the network oscillation can reduce the number of bursts observed, a shift in the dynamics of pyramidal cells towards increased propensity to burst via GABA_B feedback can overcome this interference and restore coding through burst fraction. We conclude that although the inclusion of GABA_B feedback is deleterious to linear coding, it is able to restore some portion of the normal ability to burst in response to the specialized stimulus elements (e.g. low frequency components of the broadband input), which recruit bursts (Doiron et al. 2007; Gabbiani et al. 1996; Mehaffey et al. 2007; Oswald et al. 2007).

5.2.5 The increase in bursting invoked by GABA_B feedback improves feature detection and frequency-specific spike patterning.

Although GABA_B receptor activation has deleterious effects on the ability of the spike train to produce a linear estimate of input currents, we considered that it may have other beneficial effects on neural coding. In the first case we considered that ELL pyramidal cells are able to code distinct frequency content in parallel by dividing the spike train into two components: bursts of spikes that are coherent with low frequency
events, and isolated spikes that are coherent with higher frequency content (Oswald et al. 2004). It has further been shown that tonic GABA_B receptor activation can improve the segregation of frequency content into distinct ISI ranges by regulating the intrinsic burst generating mechanism (Mehaffey et al. 2007). In vivo, the GABA_B currents do not occur tonically, but instead arise from the nP bipolar cell feedback pathway. This feedback is recruited primarily by the same GABAergic projections which cause a network mediated gamma oscillation via the GABA_A conductance (Doiron et al. 2003a; Doiron et al. 2004). We used the network model in order to examine the interaction between these two effects of the network feedback: a GABA_A component involved in the creation of a gamma oscillation, and a GABA_B component which can regulate the intrinsic bursting due to the intrinsic oscillatory dynamics of each cell. When the network is driven with uncorrelated sensory inputs, the resulting coherence between the stimulus and components of the pyramidal cell spike train (e.g. bursts and isolated spikes) resembles that observed previously (Mehaffey et al. 2007; Oswald et al. 2004) in that bursts of spikes are coherent with low frequency stimulus components. Isolated spikes show a small preference for high frequency stimulus components but are largely broadband in their frequency preference (Figure 5-7A). When the system is presented with correlated inputs in a model network where all feedback is GABA_A mediated, the low frequency stimulus-response coherence is reduced for both bursts and isolated spikes (Figure 5-7B). As described above, a ~30 Hz oscillation will interfere with the ability of low frequency stimulus components to generate bursts, whereas high frequency components may be better able to escape such interference. Thus, the burst/low-frequency coherence is reduced, effectively increasing the overlap between the stimulus-response coherence for bursts and for
Figure 5-7 GABA_B mediated feedback regulates parallel coding of distinct frequency ranges as spikes or bursts.

Stimulus-response coherence of pyramidal cell spike output in the presence or absence of nP GABAergic feedback. A) When the system is driven by uncorrelated inputs without incorporating GABAergic feedback, the preference for low frequency events to be coded by bursts of spikes is apparent, while higher frequency events are coherent with isolated spikes. B) By including GABA_A feedback, and the resulting network oscillation, low frequency coherence in both the isolated spike train and in the burst train is reduced for correlated inputs. Although the bursts are still coherent with low frequency events, there is a greater overlap in the frequency range coded by bursts and isolated spikes. C) Including GABA_B feedback reduces the low frequency isolated spike coherence to correlated inputs, such that bursts are again preferentially responsive to low frequency stimulus events in comparison to isolated spikes.
isolated spikes (Figure 5-7B), such that there is no longer any frequency preference. Inclusion of GABA_B-mediated feedback does not increase the low frequency coherence in response to bursts. However, the GABA_B mediated component is able to reduce the coherence between isolated spikes and low frequency events, consistent with previous reports (Mehaffey et al. 2007). This then allowed low frequencies to again be preferentially encoded by burst events, even though there is a decrease in low-frequency coherence (Figure 5-7C). Increasing the relative capacity for bursts to code for low-frequency events is thus one benefit of incorporating GABA_B mediated feedback. A second way we considered that the increased bursting might be advantageous was to consider the electrosensory system as operating as a feature detector, rather than as a linear encoding device. Indeed, in the electrosensory system, burst of spikes have been suggested to code preferentially for features rather than being optimized for stimulus reconstruction (Gabbiani et al. 1996; Metzner et al. 1998). In order to analyze the ability of our model cells to detect features of afferent input we calculated the optimal feature, defined as the best linear discriminator between spike events and null events (e.g. events that fail to trigger a spike). In practice, for intracellular current injections this will generally be an upstroke of the stimulus potential, as it is in this case. We then projected the stimulus evoking each event (burst, isolated spike, or non-spike) onto this optimal feature. The resulting probability distributions define how well a class of events match the optimal feature (Figure 5-8A). These probability distributions were then used to calculate the receiver-operator characteristic (ROC) curve for bursts of spikes, as well as for the individual, isolated spikes.
Figure 5-8 ROC analysis reveals improved signal detection with GABA<sub>B</sub> feedback.

A-C) Probability densities describing the projection of events causing a null response (black), an isolated spike (green) or a burst of spikes (red) onto the optimal feature. Each plot illustrates the probability distribution for a class of events (bursts, spikes, and null (e.g. non-spike)) events projected onto the optimal feature (A), correlated inputs without GABA<sub>B</sub> (B) and correlated inputs with GABA<sub>B</sub> (C) feedback. D) ROC analysis of feature detection by bursts and isolated spikes. Under uncorrelated input conditions (dashed lines), bursts (red) outperform isolated spikes (green), consistent with previous results (Gabbiani and Metzner 1999; Gabbiani et al. 1996). During the network mediated oscillation, but in the absence of GABA<sub>B</sub> feedback, the performance of both bursts and isolated spikes deteriorates (dotted lines). Increasing the probability of bursting through GABA<sub>B</sub> in the feedback system for the network can restore some of the lost performance (solid lines).
From this analysis we observed that when the network was presented with uncorrelated inputs, individual pyramidal cell models were able to accurately detect their optimal features with bursts of spikes, whereas individual spikes performed less reliably at detecting the feature, consistent with results obtained \textit{in vivo} (Gabbiani and Metzner 1999; Gabbiani et al. 1996). When presented with correlated inputs, the performance of these cells deteriorated because a subset of these stimulus features failed to evoke spiking (bursts or isolated spikes). This occurs obligatorily because of the independent (e.g. not stimulus related) nature of the inhibition due to the network-mediated oscillation. Thus, events that should have generated a spike will fail to do so if inhibition occurs simultaneously with the stimulus event. The GABA_B-mediated burst regulation can serve to improve this detection in two ways. First, a degree of global inhibition will shunt the response to the weak inputs that provide many of the largest deviations from the optimal feature. Second, a more reliable recruitment of bursts by low frequency stimulus components will make the response to slow inputs (e.g. bursts) more reliable, with a concomitant decrease in the tendency to fire isolated spikes in response to slow input. This again will narrow the repertoire of responses which are then compared to the optimal feature. Obviously a sharpening of the frequency selectivity and a greater similarity to an optimal feature will covary, and these two mechanisms are therefore complementary, if not directly interdependent.

When we examine the resulting ROC curves (Figure 5-8B), the incorporation of GABA_B feedback (and regulation of burst firing) improves signal detection by both bursts and isolated spikes. As such, the regulation of bursting by GABA_B receptor activation is able to correct for some of the deficiencies induced by the network feedback.
Although the coding fraction, and therefore the ability to accurately reconstruct the
stimulus based on the firing pattern of the pyramidal cells, remains depressed, the ability
to detect specific signals is maintained, while the mapping of frequency to distinct spike
patterns is improved. Given the suggestion that bursts code for low frequency, prey-like
inputs acts as reliable feature detectors (Gabbiani et al. 1996; Oswald et al. 2004; Oswald
et al. 2007) the data presented here may indicate a mechanism for the disambiguation of
conspecific signals from prey and environmental signals.

5.3 Specific Discussion

Electric fish rely heavily on their electro sensory system in order to parse
broadband signals relating to a complex sensory environment. This sense is used to detect
prey and environmental signals (MacIver et al. 2001), establish social order
(Heiligenberg 1973), and to communicate between conspecifics (Zupanc et al. 2006). As
such, it represents a major sensory system for these animals that is used for a variety of
purposes. The problem of how neurons are able to parse a given stimulus in order to
selectively encode distinct features of the sensory environment remains an outstanding
problem in neuroscience. The distribution of components of the sensory scene into
distinct spike trains provides one possible mechanism for accomplishing such a task (see
General Introduction, Chapter 1). Here, I have examined the interaction between an
intrinsic burst mechanism, which is known to be required for the parsing of frequency
content in the spike trains (Oswald et al. 2004), and a feedback mediated network
oscillation recruited under a specific behavioural context. This feedback from the nP
contains two components. The first, a 30 Hz GABA_A mediated inhibition, creates a network oscillation. The second, a relatively tonic GABA_B mediated component, regulates the burst mechanism intrinsic to each cell.

5.3.1 Involvement of the intrinsic burst mechanism

Spike bursts in ELL pyramidal cells have a well established intracellular mechanism that relies on a spatially distributed interaction between somatic and dendritic spike discharge (Fernandez et al. 2005b; Lemon and Turner 2000; Turner et al. 1994). In terms of sensory processing in *Apteronotus* this mechanism allows for the segregation of high and low frequency stimulus content into distinct ranges of ISIs that reflect the generation of spike bursts or isolated spikes (Oswald et al. 2004). This allows a separation of low and high frequency stimulus components, which correspond to environmental/prey signals and communication signals, respectively (MacIver et al. 2001; Oswald et al. 2004; Zupanc et al. 2006). This dependence on a soma-dendritic interaction to create the firing dynamics of pyramidal cells further allows spatially distributed inputs to regulate the firing behaviour. In the previous chapter, we showed that a GABA_B mediated delay in the backpropagating dendritic spike serves to decrease burst threshold and sharpen the frequency tuning of the cell (Mehaffey et al. 2007) (Chapter 4). In this chapter we examined the interaction between the regulation of the intrinsic dynamics of burst discharge in pyramidal cells by GABAergic synaptic feedback and the network oscillation which necessarily coincides during feedback activity from the nP.
5.3.2 Oscillations and coding

Gamma frequency oscillations have been observed in many systems, and are heavily modulated by contextual situations in several mammalian brain regions (Buzsaki 2006). Although much effort has been expended in both computational and experimental studies to examine mechanisms for generating oscillations (Borgers et al. 2005; Cunningham et al. 2003; Gloveli et al. 2005), their implications for sensory processing and signal discrimination are only recently beginning to be understood (Borgers and Kopell 2007; Doiron et al. 2003a; Masuda and Doiron 2007). We examined here a network mediated gamma oscillation in a model of the nP-ELL feedback pathway. This oscillation is created by the recruitment of delayed inhibition when the network is driven by correlated inputs (Doiron et al. 2003a; Doiron et al. 2004). In electric fish the ELL is most likely to receive correlated inputs when a large number of receptive fields are driven by spatially coherent inputs to electroreceptors (Doiron et al. 2003a). This occurs primarily in social situations (e.g. 2 or more fish), where the electric field of a conspecific influences a population of electroreceptors distributed over the surface of the animal. Importantly, such communication calls are characteristically high frequency (Zupane et al. 2006), and a decrease in the low-frequency response due to the inhibitory oscillation may shift the ability to attend to high-frequency inputs. Specifically, since gamma-frequency inhibition attenuates the low-frequency component of the stimulus-response coherence with little effect on higher frequency content, there may be a bias in the resulting spike trains toward communication signals.
Although there are contexts in which gamma oscillations can be shown to aid in coding (Borgers and Kopell 2007; Masuda and Doiron 2007), we have shown that in terms of an accurate representation of a sensory input, these oscillations can have deleterious consequences. Similar results have been obtained *in vivo* where the coding fraction of ELL pyramidal cells was significantly reduced by global stimulation and the resulting oscillations (Doiron et al. 2003a). As these oscillations are mediated by feedback inhibition, it is expected to prevent the occurrence of spikes. Because this inhibition is independent of the stimulus, it will in turn prevent some stimulus upstrokes from generating spikes. This necessarily reduces the accuracy of the representation of the stimulus by the spike train, as some events will not be coded.

The ability to select for specific features also deteriorated in the presence of the delay-induced oscillation, but could be restored through a GABA\textsubscript{B} mediated feedback regulation of the intrinsic burst generating mechanism (Mehaffey et al. 2007). The question remains however, whether the gamma oscillations that arise through delayed feedback provide any significant advantage to signal processing. We show here that in the electrosensory system, gamma oscillations can preferentially reduce low frequency coherence, which in turn may reduce the ability of spikes to code low frequency inputs. Such a shift would be predicted to help accentuate the high frequency inputs that should occur in the presence of conspecifics. However, there remains a low frequency component to the response, as well as to the electrosensory environment. The resulting stimulus-response coherence during the gamma oscillation can contain representations for low-frequency inputs encoded as both bursts and spikes. This creates an ambiguity, as normally bursts are the preferential coders of low-frequency inputs in these cells (Ellis et
al. 2007b; Mehaffey et al. 2007; Oswald et al. 2004). However, regulation of the burst mechanism by a GABA\textsubscript{B} mediated conductance was able to create a greater preference for representing low frequency inputs by bursts. This in turn led to a greater segregation of the encoding of specific frequency bands by distinct patterns of spikes (e.g. bursts and isolated spikes). Such a segregation of the surrounding scene into subcomponents is reminiscent of figure-background segregation, where the visual system must separate incoming inputs into meaningful groups (Spillmann 2006).

We thus show though a simple network model that GABA\textsubscript{B} feedback regulation of pyramidal cell burst dynamics in the face of a network mediated oscillation can allow for a more complete segregation of the spike train into distinct frequency bands, and in turn a more specialized processing of environmental inputs of distinct classes.
Chapter Six: Intrinsic frequency tuning in ELL pyramidal cells varies across electrosensory maps.

6.1 Introduction

The complex and multi-dimensional nature of sensory stimuli often contains more information than a single neuron can accurately represent in a spike train. In order for the nervous system to encode important characteristics of the outside world, sensory neurons must initially respond to specific aspects of the sensory input. One component of natural signals commonly encoded by sensory neurons is frequency (either spatial or temporal), which has been shown to be important for visual (Butts et al. 2007; Lesica and Stanley 2004), auditory (Fritz et al. 2003; Woolley et al. 2005), somatosensory (Luna et al. 2005; Romo et al. 2004) and electrosensory (Chacron et al. 2003; Gabbiani and Metzner 1999; Oswald et al. 2004; Shumway 1989a) processing. The ability of a cell to respond to specific frequencies within a broadband signal implies mechanisms for frequency tuning. Such frequency tuning can be implemented by intrinsic conductances and cell dynamics (Ellis et al. 2007b; Izhikevich 2002), by feedforward synaptic dynamics (Zhang et al. 2003), short-term synaptic plasticity (de la Rocha and Parga 2005), and feedback from higher brain centers (Chacron et al. 2003; Chacron et al. 2005).

As reviewed in Chapter 1, the ELL is divided into four topographic maps of the body surface termed the medial (MS), centromedial (CMS), centrolateral (CLS), and lateral (LS) segments (Bell and Maler 2005). Pyramidal neurons of the CMS, CLS and LS receive primary afferent input from tuberous receptors that detect perturbations in the amplitude of the EOD (Figure 6-1A,B). Upon entering the ELL, primary afferent fibres
trifurcate and provide identical tuberous receptor input to each of the three tuberous
segments (Carr et al. 1982; Heiligenberg 1991b). The neural circuits in each of the three
tuberous segments are also morphologically similar and function essentially as repeating
topographical maps with no inter-map connections. Such patterns of distinct maps within
a sensory modality are common in sensory systems, and have been shown to allow
parallel processing of different types of information (Metzner 1999; Schreiner and Winer
2007; Young 1998). In the ELL, pyramidal cells within these three segments show
different preferences for the frequency content of input signals. Cells in the more medial
segments prefer inputs at lower frequencies while the more lateral segments prefer higher
frequencies in \textit{in vivo} studies (Krahe et al. 2008; Shumway 1989a). The differential
frequency tuning between maps correlates to specific electrosensory behaviours: more
medial segments process low frequency-related input associated with a “jamming
avoidance response”, and lateral segments the communication behaviours involving
higher frequency inputs (Metzner and Juranek 1997). Furthermore, \textit{in vivo} recordings
have established a frequency preference between E- and I-cells in the CLS and LS maps,
with E-cells exhibiting tuning to higher frequencies and exhibiting greater regulation of
frequency tuning than I-cells (Chacron et al. 2005; Krahe et al. 2008). These results are
also consistent with observations \textit{in vitro} that intrinsic membrane properties of the E- and
I-cell class are biased towards different frequency ranges (Ellis et al. 2007b).

As ELL pyramidal cells show frequency selectivity to broadband inputs both \textit{in}
\textit{vitro} (Ellis et al. 2007b) and \textit{in vivo} (Chacron et al. 2003; Chacron et al. 2005), they
present an ideal model to identify the cellular and synaptic basis of frequency tuning.
Figure 6-1 Distribution of spike bursts across pyramidal cell types and ELL maps.

A) The ELL is divided into four segmental maps; three segments (CMS, CLS, LS) receive tuberous inputs which code amplitude information. Each primary afferent (black) trifurcates, resulting in a topographic representation of its receptive field in each of the three maps. B) Each afferent gives inputs to both E- and I-type pyramidal cells. E-cells receive excitatory afferent inputs directly onto their basal dendrites and are excited by increases in EOD amplitude. I-cells receive input through inhibitory granule cells II (GC2) and fire in response to decreases in EOD AMs. C) Representative traces of bursting in I-cells only in each of the three ELL segments in response to step intracellular current injections. bAHPs are indicated by arrows. D) Fraction of E- and I-cells exhibiting burst discharge in each segment. Bursts are more common in I- than E-cells within each map (asterisks). E) The amount of current required to evoke the transition from isolated spiking to bursting (e.g. the rheobase for bursting relative to spike threshold) is significantly lower in I-cells of the LS compared to CLS and CMS maps. Note that this was only calculated for I-cells as too few bursting E-cells were observed in the more lateral segments (see (D) for statistical comparisons.)
across multiple sensory maps in the CNS. The contribution of intrinsic cellular mechanisms to differential frequency tuning remains to be determined. One promising line of investigation has examined the detection of input frequency by bursts of spikes. Indeed, the segregation of bursts and single spikes to encode distinct AM frequency ranges has been observed in electric fish (Doiron et al. 2007; Gabbiani et al. 1996; Oswald et al. 2004; Oswald et al. 2007) and in the mammalian visual (Lesica and Stanley 2004; Lesica et al. 2006) and auditory systems (Eggermont and Smith 1996). Bursts have also been shown to be more reliably timed to stimulus features, to show superior feature detection properties, and to more reliably activate downstream cells than can single spikes (Gabbiani et al. 1996; Izhikevich et al. 2003; Kepecs and Lisman 2003; Lisman 1997; Metzner et al. 1998).

The current chapter focuses on the intrinsic membrane properties of E- and I-cells to identify any correlates of membrane physiology that could account for differential frequency tuning between these cell types and across ELL sensory maps. Extensive work on ELL pyramidal cells in vitro and in vivo has identified the role and significance of an intrinsic soma-dendritic interaction involving a conditional backpropagation of dendritic spikes in the generation of burst discharge (Turner et al. 1994). Moreover, this work has shown that pyramidal cell bursts are important for feature detection of sensory stimuli, and particularly for low frequency components of a stimulus (Mehaffey et al. 2007; Metzner et al. 1998; Oswald et al. 2004; Oswald et al. 2007). The ability of pyramidal cells to encode low frequency events with bursts is under significant regulation by intrinsic conductances (Ellis et al. 2007b; Rashid et al. 2001b), synaptic input (Mehaffey et al. 2007), and neuromodulatory agents (Ellis et al. 2007a) that can affect the
underlying soma-dendritic interaction. Indeed we have previously shown that burst
discharge and frequency tuning can be affected by the properties of pyramidal cell spike
discharge. For instance, a high threshold potassium current expressed in pyramidal cells
allows high frequencies of spike discharge (Fernandez et al. 2005a). Increases in somatic
spike width can alter bursting by reducing the mismatch between somatic and dendritic
voltage that underlies a DAP involved in producing spike bursts (Fernandez et al. 2005b).
An apamin-sensitive AHP is involved in creating the frequency tuning profile of
pyramidal cells (Ellis et al. 2007b), while a theoretical study has further shown that the
adaptation rate of pyramidal cell discharge is capable of regulating low frequency tuning
(Benda and Herz 2003). Inasmuch as these parameters of spike discharge could be
regulated in pyramidal cells, they could contribute to establishing differential frequency
tuning across sensory maps.

In this chapter we tested the hypothesis that differential frequency tuning by
pyramidal cells across ELL sensory maps involves the regulation of membrane properties
that underlie spike generation and bursting, and their adaptation rates in response to
quasi-naturalistic stimuli. In fact, we find that several fundamental aspects of ELL
pyramidal cell spike waveform differ across maps in a manner that can contribute to the
creation of frequency tuning.

6.2 Specific methods

A total of 118 recordings were obtained from pyramidal cells in all three ELL
segments receiving tuberous inputs (samples sizes for CMS: 44, CLS: 47, LS: 27). The
map in which recordings were made was identifiable as the borders between maps are clearly defined in the *in vitro* slice. Recordings were digitized at 10-40 KHz using a NI PCI-6030E DAQ board (National Instruments, Austin, TX) and recorded in custom software using the Matlab data acquisition toolbox (Mathworks, Natick, MA). Cells were held at a level just below firing threshold using negative current injection. Random amplitude modulations (RAMs) consisting of white noise low-pass filtered to contain power between 0-60 Hz. RAM stimuli were provided in addition to the bias current and the SD of the RAM was adjusted to give average firing rates of 10-30 Hz, as at these frequencies the estimated coherences were reliable in their frequency preferences. Recordings were rejected if any part of the stimulation protocol could not be performed.

### 6.2.1 Cell fills

Pyramidal cells were filled with Neurobiotin using a positive current ejection pulse (+1 nA, 2 Hz). Following recording slices were transferred to 4% paraformaldehyde and fixed for several days at 4°C. Slices were then washed in 0.1 M phosphate buffer (PB) for several hours and placed in a solution of PB, Triton X-100 (0.1%), DMSO (0.5%) and streptavidin-Cy3 (1:1500) for three days. Slices were then slide-mounted for visualization on an Olympus BH-2 research microscope to identify filled cells according to E- or I-cell, position within the ELL PCL (superficial, intermediate or deep), and segment (CMS, CLS or LS). Images were taken using Fluoview software on an Olympus FV300 BX50 confocal microscope. Of 118 cells recorded from the three maps, 43 were successfully filled with Neurobiotin. Superficial
pyramidal cells were distinguished by the extent of their dendritic arborization and by location of the soma within the cell layer (Bastian and Courtright 1991; Bastian and Nguyenkim 2001). Cells were classified as E- or I-cells depending on the presence or absence of a basilar dendrite, or by their frequency preference (Ellis et al. 2007b). Of the filled cells classified following Neurobiotin injection, 8 were from the LS, 11 from the CLS, and 24 from the CMS.

Spike characteristics were measured at the weakest magnitude of DC current injection that elicited spiking and analysed with custom software in MatLab. AHP measurements were taken as the minimum voltage between spikes. Spike threshold was assessed based on the derivative of the voltage trace, and defined as the initial value eight times greater than the standard deviation of the subthreshold noise. The level of current injection required to elicit bursting was assessed by visual inspection. Burst threshold was then defined as the amount of current injection required to transition from initial tonic spiking into bursting (Mehaffey et al. 2007; Noonan et al. 2003). Cells were classified as non-bursting if they reached a saturating firing frequency but did not burst, or if more than 0.6 nA of current was injected above spike threshold without eliciting bursting. Gain was determined from the slope of a linear fit to the FI curve. Adaptation time constants were fit to 100 s RAMs with spike trains binned into 1 s segments. Statistical significance was assessed using a 1-way ANOVA with a significance level of p < 0.05 unless otherwise noted, with post-hoc analysis using Tukey’s HSD. Average values are presented as mean ± SEM.
6.2.2 Classification of cell types based on coherence

As we record primarily from the PCL, we are sampling from populations of intermediate and superficial pyramidal cells and were unlikely to have recordings from deep pyramidal cells positioned adjacent to or within the granule cell layer (Bastian et al. 2004; Bastian and Courtright 1991). Further identification of pyramidal cell class on the basis of firing properties to square wave current injection protocols has traditionally been difficult in vitro, requiring the filling and labelling of cells for histological analysis (Ellis et al. 2007b). In contrast, during in vivo recordings the response to increases in EOD amplitude can be quickly tested and the cell identified as corresponding to either the E- or I-cell class. Our recent work established that a time-varying stimulus can also be used to distinguish E- and I-type pyramidal cells in vitro in terms of the frequency content of their response (Ellis et al. 2007b). Histologically identified I-cells in all segments were found to be ubiquitously low-pass in their frequency responsiveness, while identified E-cells in the CLS and LS were either broadband or high-pass in frequency responsiveness. In the CMS, superficial E-cells could deviate from this pattern in exhibiting low-pass responsiveness (Ellis et al. 2007b). Each of these findings were confirmed in the present study, with low-pass frequency responsiveness defined as coherence ratios (C(30-50 Hz)/C(0-20 Hz)) within 2 standard deviations above the average for anatomically identified I-cells (upper cut-off of 0.86). No anatomically filled E-cells in the CLS or LS fell into this range. Although this does not guarantee the absence of misclassifications, it suggests such errors are rare.

The remaining cells were then subdivided into a class of broadband and high-pass
cells by a coherence ratio of 0.87 to 1.19 (broadband), or a coherence ratio above 1.19 (displaying an apparent preference for higher frequencies, see Figure 6-6A and accompanying results). These two cell classes were statistically significantly different from each other and the low pass category, establishing them as separate phenotypes. The E-cell group also included any basilar pyramidal cells in the CMS which had been successfully labelled regardless of frequency preference. We note that in the CMS, some E-cells (superficial pyramidal cells) share similar frequency tuning characteristics with I-cells. Therefore our group of E-like CMS pyramidal cells is comprised of intermediate basilar pyramidal cells along with superficial cells directly identified by intracellular fills. Further, our low pass cell population may include a small number of superficial E-type CMS pyramidal cells which display low-pass characteristics (Ellis et al. 2007b). Given that the most important distinction is revealed to be frequency tuning any such errors are unlikely to affect the general conclusions of the study.

6.3 Results

One well-studied characteristic of ELL pyramidal cells is a specific pattern of burst firing, consisting of a gradually increasing rate of firing followed by a brief ‘doublet’ of spikes with a short ISI (< 10 ms) (Fernandez et al. 2005b; Lemon and Turner 2000; Turner et al. 1994) which can be observed both in vivo and in vitro (Oswald et al. 2004). We began by studying the distribution and characteristics of this burst discharge. During step depolarizations, one can detect a ‘slow burst dynamic’ as a progressively increasing firing frequency terminated by a fast ISI (the ‘doublet’). This dynamic is
driven by a frequency-dependent increases in the peak latency and duration of dendritic spikes that augments dendro-somatic current flow to enhance the somatic depolarizing afterpotential (Fernandez et al. 2005b). The short ISI of the spike doublet terminates the burst when it falls within the dendritic refractory period to block dendritic backpropagation. During in vitro recording, we observed burst firing in pyramidal cells of all three tuberous maps, with representative examples of burst discharge from each map shown in Figure 6-1C for square wave current pulses. We were able to classify E- or I-cells in the CLS and LS using a recently established methodology based on their response to broadband inputs (Ellis et al. 2007b), and through intracellular labelling (CMS: 23, CLS: 11, LS: 8). Specifically, the stimulus-response coherence shows a peak at low frequencies in I-cells while E-cells rarely show a preference for low frequencies. We thus used this distinction to classify cells when intracellular labelling was unsuccessful (see Methods) and were able to identify unlabelled I-cells in each of the three maps (CMS: 35, CLS: 31, LS:13) as well as E-cells (CMS: 9, CLS: 16, LS: 14) by this criteria.

6.3.1 Expression of burst discharge in response to DC steps across tuberous maps and cell types

We found significant differences in the fraction of E- and I-cells that displayed burst firing in each of the tuberous maps (Kruskal-Wallis 1-way ANOVA, p < 0.05). I-cells showed the greatest propensity to burst, with the majority of I-cells in each map displaying bursting (CMS: 86%, CLS: 82%, LS: 70%) (Fig. 1D). In contrast, bursting was less prevalent in E-cells in all maps, with the smallest proportion of bursting cells
apparent in the LS (CMS: 22%, CLS: 25%, LS: 7%) (Figure 6-1D). The incidence of bursting in E-cells proved to be significantly lower from that of I-cells regardless of map (p < 0.05, Mann-Whitney U). We then proceeded to examine the rheobase for burst firing, taken as the difference between the DC current injection required to evoke a burst and that required to evoke initial tonic spiking. Burst rheobase was characterized exclusively in I-cells as the low number of E-cells that displayed bursting precluded any significant statistical power. Burst rheobase was almost identical between I-cells of the CMS and CLS (0.22 ± 0.02 nA, 0.22 ± 0.03 nA, respectively, p > 0.05), but was significantly lower in the LS (0.085 ± 0.014 nA, p < 0.05) in comparison to the other two maps (Figure 6-1E). As ELL pyramidal cells appear to be maintained near spike threshold, this suggests that LS I-cells in vivo will have a lower threshold for bursting relative to I-cells in the other maps, although adaptation, threshold, differences in the convergence of synaptic input, and other processes (see below) may also have an influence.

6.3.2 Expression of burst discharge in response to time-varying inputs.

We extended our examination of burst firing by characterizing the response to time-varying inputs that more accurately simulate the input pyramidal cells receive in vivo. It has been established previously that pyramidal cell membrane voltage can accurately encode 0-60 Hz RAMs presented in vivo (Chacron et al. 2003; Middleton et al. 2006). We have therefore been able to use intracellular RAM current injection in vitro to mimic driving currents that result from external field RAMs in vivo (Ellis et al. 2007b; Mehaffey
et al. 2007; Oswald et al. 2004; Oswald et al. 2007). Briefly, we adjust the contrast amplitude of a 100 s, 0-60 Hz RAM intracellular current stimulus to give an average 10-30 Hz firing rate, similar to the firing frequencies recorded in vivo during external RAM stimuli (Chacron et al. 2003; Chacron et al. 2005; Doiron et al. 2003a).

Bursts evoked by time-varying inputs require the positive dendro-somatic feedback known to be generated by backpropagating apical dendritic spikes (Oswald et al. 2004), but in response to RAMs, bursts are more often truncated to spike doublets, either through dendritic failure or the rapid hyperpolarizations induced by high frequency variations of the signal (Doiron et al. 2007). In fact, the dendrosomatic feedback is present even in cells which do not undergo the slow burst dynamic in response to DC current steps (Mehaffey et al. 2005). As a result of this spike-dependent feedback time-varying inputs invoke a bimodal ISI histogram that can be divided into bursts and isolated spikes regardless of their ability to burst in response to DC current injections. We therefore parsed the resulting spike trains into bursts (typically spike doublets) and isolated spikes, and separately computed the stimulus-response coherence for these events (Figure 6-2A). No significant difference was noted in the ratio between bursts and spiking events (e.g. the burst fraction) between I-cells of the CMS, CLS, and LS and that of E-cells of the CMS (I-CMS: 0.221 ± 0.02, I-CLS: 0.218 ± 0.02, I-LS: 0.244 ± 0.04, E-CMS 0.191 ± 0.02, p > 0.05) (Figure 6-2B). However, burst fraction did show a significant reduction in E-cells in both the CLS (0.135 ± 0.02) and LS (0.077 ± 0.02, p < 0.05). This suggests that the medio-lateral decrease in the propensity for E-cells to burst in response to step current inputs (Figure 6-1D) is also reflected in the response to time-varying inputs (Figure 6-2B).
Figure 6-2 Response of pyramidal cells to time-varying inputs.

A) Representative recording (top trace) from an I-cell of the CLS showing the patterns of bursts (arrows) and isolated spikes evoked in response to a time-varying intracellular current injection (RAM, lower trace). B) Ratio of burst events in comparison to all spike events (burst fraction) evoked in response to time-varying stimuli. The total number of observed bursts decreases from medial to lateral segments in E-cells but not I-cells. C) Fraction of the low frequency (0-20 Hz) coherence encoded by bursts. A large fraction is encoded in all I-cells and CMS E-cells, but a smaller fraction is encoded by CLS and LS E-cells. D) Representative ISI densities for I-cells. Across maps, the time at which the nadir of the bimodal ISI histogram occurs is conserved. (E) Representative ISI densities for E-cells, showing that the nadir of the bimodal ISI histogram is conserved. Further, the number of events classifiable as burst events (e.g. the left peak) decreases from CMS to LS segments. Arrows denote the nadir of the histogram used to distinguish short ISIs (e.g. burst events).
It has been shown that bursts of spikes code preferentially for low frequency events, while isolated spikes show a broadband or high-pass frequency preference (Mehaffey et al. 2007; Oswald et al. 2004). The selective encoding of low frequency inputs by bursts of spikes both requires and is regulated by the intrinsic burst mechanism (Ellis et al. 2007b; Mehaffey et al. 2007; Oswald et al. 2004). In order to examine the relationship between low frequency events and bursts of spikes, we quantified the fraction of the total 0-20 Hz coherence encoded by bursts of spikes. We found that this measure closely followed the pattern observed in the burst fraction (Figure 6-2B), where I-cells of all segments had the greatest ability to encode low frequency events with bursts of spikes (CMS: 0.52 ± 0.03, CLS: 0.53 ± 0.04, LS 0.53 ± 0.08, p > 0.05). E-cells of the CMS were also able to encode low frequency events (CMS: 0.47 ± 0.04) at a level that was statistically indistinguishable from I-cells of all segments (Figure 6-2). In comparison, E-cells of the CLS and LS showed a reduced tendency to encode low frequency inputs with bursts of spikes (CLS: 0.32 ± 0.04, LS 0.24 ± 0.04, p < 0.05). Taken together, these data suggest that the degree to which ELL pyramidal cells encode low frequencies with bursts varies across the maps (for E-cells) and cell types (E- vs. I-cells). The cells with the greatest tendency to express the slow burst dynamic (e.g. Figure 6-1D) also show the strongest ability to encode low frequency inputs using bursts. This occurs despite the fact that the positive dendro-somatic feedback required to establish the ISI histogram bimodality (Oswald et al. 2004) occurs even in cells which do not express these slow burst dynamics (Mehaffey et al. 2005). Although in response to brief DC inputs, LS I-cells show a lower burst threshold, in response to long lasting RAMs they only burst as
often as I-cells in other segments. This may be due to the adaptation observed over long periods of stimulation (see below). Such adaptation implies that the lower threshold for bursting in LS I-cells would be observed primarily in response to low frequency transients larger than the average background fluctuations to which it has adapted.

We plot representative ISI histograms for both I-cells and E-cells in Figure 6-2D,E using a log scale for time to clearly show the bimodality (Ellis et al. 2007b; Turner et al. 1996). Previous studies have suggested that the nadir of the ISI histogram is highly conserved in the *in vitro* preparation (Ellis et al. 2007b; Mehaffey et al. 2007; Oswald et al. 2004; Turner et al. 1996). As described above, all pyramidal cells regardless of map or cell class, showed a clear bimodality with a nadir near 10 ms (8-10 ms, $p > 0.05$ across cell types and all maps), establishing ISIs < 10 ms as our burst ISI criterion. Note that the lower density of ISIs in the first peak of the ISI histogram in E-cells of the CLS and LS are indicative of the lower burst fraction observed in these cells. A previous study examined both differences between maps and between E- and I-cells, and found that I-cells were better feature detectors, and that the CMS as a whole outperformed the LS at feature detection, particularly when bursts were analysed (Metzner et al. 1998). The differences in bursting both between cell classes and across maps may have contributed to this result, particularly as the frequency cutoff for their RAM stimulations was between 2-40 Hz, a frequency range where bursts are expected to play an important role (Doiron et al. 2007).
6.3.3 E- and I-cells differ in spike properties

We initially began by testing the hypothesis that the complement of ionic currents differed between E- and I-cells, as recently found for the SK current (Ellis et al. 2007b). We grouped all the identified E-cells (n = 39) and I-cells (n = 79), and examined various parameters of evoked spikes to establish whether there were any significant differences between the two cell classes. We examined the average characteristics of the spike waveform at the minimal current injection sufficient to induce repetitive firing. E- and I-cells were significantly different in all of three key parameters examined (Figure 6-3A-C), including the magnitude of the AHP (I-cells: 6.18 ± 0.33, E-cells: 7.42 ± 0.41 mV, p < 0.05) the spike half-width (I-cells: 0.618 ± 0.019 ms, E-cells: 0.809 ± 0.03, p < 0.05), and the voltage threshold for spiking (I-cells: -66.57 ± 0.74 mV, E-cells: -62.79 ± 0.74 mV, p < 0.05). Interestingly, the differences in voltage threshold for spiking may optimize a pyramidal cell’s response to the inputs they receive. E-cells receive direct excitatory input from electroreceptors, while I-cells translate the EOD primarily through the removal of disynaptic inhibition. A lower spike threshold in I-cells may then contribute to a higher sensitivity to afferent input. The factors determining threshold remain unknown, but could involve low-threshold potassium currents known to be expressed in ELL pyramidal cells (Ellis et al. 2007a; Fernandez et al. 2005a; Mathieson and Maler 1988; Mehaffey et al. 2006; Smith et al. 2006). The larger AHP in E-cells may be related to the recently established preferential expression of somatic SK2 conductances in this cell class (Ellis et al. 2007b).
Figure 6-3 E- and I-cell populations are significantly different with respect to spike properties.

Plots of the mean value of different spike parameters between E- and I-cells. On average E-cells exhibit a significantly wider spike (A), a larger magnitude AHP (B), and a higher spike threshold (C) than I-cells. Sample sizes used to calculate histograms in (A-C) are shown in brackets in (C).
6.3.4 Spike characteristics show only minor differences across maps

A number of studies have found differences in the expression pattern of specific potassium channels across the tuberous maps of the ELL, which generally increase in labelling intensity along a mediolateral axis (Deng et al. 2005; Ellis et al. 2007b; Mehaffey et al. 2006; Rashid et al. 2001a; Smith et al. 2006). Gradients of ion channel expression in the auditory system have been shown to underlie differences in the spikes generated, which in turn can contribute to specialized neuronal computations (Brew and Forsythe 2005; Li et al. 2001; Parameshwaran et al. 2001; Rosenblatt et al. 1997; von Hehn et al. 2004). We therefore examined spiking characteristics to see if gradients of channel expression in the ELL translate into differences in the spike waveform, and in turn, frequency tuning across maps.

We hoped to find spike parameters that varied with cell type and map as suggested by the distribution of potassium channels. Ideally, these characteristics would, like coherence, provide an electrophysiological signature to identify cells without histological analysis. We began by examining I-cells (low-pass). Representative examples of spikes recorded from I-cells from each of the three maps are shown in Figure 6-4. When these I-cell spikes were superimposed little variability was detected (Figure 6-4B), as supported by the lack of significant differences for various spike parameters between I-cells in each of the three maps. Spike half-width was nearly identical in I-cells between the three maps (CMS: 0.623 ± 0.035 ms, CLS: 0.613 ± 0.028 ms, LS: 0.617 ± 0.018 ms, p > 0.05), suggesting a similar net complement of sodium and potassium channels underlying the spike regardless of map. In support of this, there were no
Figure 6-4 Spike parameters of I and E-cells across tuberous segments.

A) Representative traces of individual spikes of I-cells recorded from each of the three tuberous segments, CMS (light grey), CLS (dark grey) and LS (black). B) Superimposition of spikes shown in (A) reveals minimal variability in spike shape. C) Average values from recordings in each map show no significant differences in I-cell spike width, AHP size, or spike threshold. D) Representative traces of individual spikes recorded from E-cells within the segments, CMS (light grey), CLS (dark grey) and LS (black). E) Superimposition of spikes shown in (D) reveals larger spike width of LS E-cells. F) Spike width increases in a medio-lateral fashion and is significantly longer in the LS segment, while no significant differences in AHP size or spike threshold is found across maps. Sample sizes for recordings in each segment used to calculate histograms in are shown in brackets.
significant differences between cells in either the magnitude of the AHP (CMS: 5.70 ± 0.385 mV, CLS: 7.13 ± 0.78 mV, LS: 6.31 ± 1.36 mV, p > 0.05) (Figure 6-4D), or the spike voltage threshold (CMS: -67.5 ± 1.08 mV, CLS: 65.3 ± 1.15 mV, LS: -67.4 ± 2.02 mV, p > 0.05) (Figure 6-4C). This suggests that I-cells are highly homogenous in their spiking characteristics across the three maps. In contrast, our examination of E-cells (as defined by the coherence criteria) showed that spike width varied significantly across maps, with LS cells having a wider spike half-width than those in either the CMS or CLS (Figure 6-4D,E); the latter were not significantly different from each other (CMS: 0.786 ± 0.028 ms, CLS: 0.744 ± 0.026 ms, LS: 0.959 ± 0.054 ms, p < 0.05). No such differences were found in AHP depths (CMS: 7.16 ± 0.75, CLS: 7.48 ± 0.64, LS: 7.45 ± 0.86, p > 0.05) or in the spike threshold (CMS: -63.4 ± 1.01 mV, CLS: -64.5 ± 1.28 mV, LS: -60.98 ± 1.06 mV, p > 0.05) for E-cells (Figure 6-4E). A recent theoretical study (J. Middleton, personal communication) suggested a computational advantage if E-cells of the LS have a higher threshold than cells in the other segments. In order to test this, we pooled the CMS and CLS cells for comparison to LS pyramidal cell threshold. This proved significant (CMS/CLS Pooled: -64.2 ± 0.8 mV, LS: -60.98 ± 1.0 mV, p < 0.05), suggesting that, as predicted, the increased threshold in LS pyramidal cells is specific to that map.

The expression of SK2 K⁺ channels varies in a map-specific fashion for E-cells, but not for I-cells (Ellis et al. 2007b). Taken together, our results suggest that other currents may follow a similar pattern, preferentially regulating the width and threshold of the spike waveform in the most lateral segment, but in a fashion exclusive to E-cells. However, the lack of a graded difference across maps in such variables as AHP depth
Figure 6-5 Frequency selectivity varies with cell type and map.

In each case representative examples of the coherence between pyramidal cell spike train and the intracellular current RAM stimulus (0-60 Hz) is shown, along with a Neurobiotin-labelled pyramidal cell. Basilar dendrites of E-cells are denoted in (B) by arrowheads. A) I-cells exhibit low-pass characteristics in preferring low frequency (0-20 Hz) over higher frequency (30-50 Hz) inputs to a similar degree across all ELL maps. B) E-cells show a clear shift in frequency selectivity across maps. Observed frequency tuning includes low-pass (CMS), broadband (CLS), and high-pass (LS) selectivities.

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does not match the expression pattern observed in immunohistochemical studies where medio-lateral gradients of expression were observed for many different channels (Deng et al. 2005; Ellis et al. 2007b; Mehaffey et al. 2006; Rashid et al. 2001a; Smith et al. 2006). We hypothesized that some heterogeneity in the computations performed by E-cells could account for these distinctions.

6.3.5 Frequency tuning characteristics vary with cell class and across maps

Having examined spike parameters, tendency to burst, and the relationship between bursting and low frequency tuning in pyramidal cells, we next considered the full spike train, including both bursts and isolated spikes. We calculated the stimulus-response coherence (for 0-60 Hz RAM used as an intracellular current injection stimulus) for cells in all three maps and for both E- and I-cells. Figure 6-5 shows Neurobiotin fills of representative cells from each map along with the associated 0-60 Hz coherence. Consistent with a previous study (Ellis et al. 2007b), labelled I-cells were low-pass regardless of map (Figure 6-5A) and displayed a clear peak in their stimulus-response coherence at low frequencies (0-20 Hz). In comparison, E-cells identified by their basilar dendrites (Figure 6-5B) exhibited peaks in the coherence at low frequencies (e.g. Figure 6-5B, CMS), a broadband frequency response (e.g. Figure 6-5B, CLS), or a preferential response to higher frequencies (Figure 6-5B, LS). Because our previous work had established that CMS E-cells can contain at least two populations, one low-pass, and one without noticeable frequency preference (Ellis et al. 2007b), we considered whether similar heterogeneities might exist within the more lateral maps. We determined the
coherence ratio for all cells (e.g. the ratio of the 30-50 Hz coherence to the 0-20 Hz coherence). This gave us an index of the frequency tuning characteristics of the cell, where high coherence ratios indicate high-pass characteristics, and numbers near unity indicate broadband characteristics (Ellis et al. 2007b; Mehaffey et al. 2007). Previous work has determined that the low-frequency tuned population of I-cells showed coherence ratios below 0.86, and this cut-off was used to separate a low-frequency responsive population (mean coherence ratio, 0.66 ± 0.01). We then considered the possibility that cells may be subdivided into those displaying an opposite frequency preference—e.g. a preference for high frequencies. An examination of the distribution of coherence ratios of the remaining cells (Figure 6-6A) allowed a further separation into a population between 0.86 and 1.19 (mean, 0.96 ± 0.01), indicative of approximately broadband tuning. The distribution also displayed a long tail of discretely clustered cells (Figure 6-6A), that we used to define cells with a preference for higher frequencies over low frequencies (1.96 ± 0.3). The mean coherence ratios for each class of cells are shown in Figure 6-6B. We then examined the distribution of frequency tuning across all three maps.

When considering the overall frequency tuning characteristics for both bursts and isolated spikes together, we found that the CMS was predominantly low-pass (Figure 6-6C), with a small number of broadband cells, previously established to be intermediate E-cells (Ellis et al. 2007b). The CLS cell population was more equitably distributed between low-pass and broadband cells (Figure 6-6C), likely reflecting the equal probability of recording from E- or I-cells. Finally, in the LS, approximately half of the cells were low-pass, consistent with approximately half the cells being I-cells. The
Figure 6-6 Frequency selectivity is distributed across maps.

A) The low-pass cells (<0.86) are not represented since their large numbers would make it difficult to discern the high pass population. Histogram showing the distribution of band and high-pass cells. The lower limit for the broadband cells was 0.86 and the upper limit 1.19 (arrow), cells above this value were considered to display a preference for higher frequencies (e.g. high-pass). Note that, for this small sample, the high-pass population occurs as discrete frequency-preferring clusters. B) Mean coherence ratios obtained for all cell types (e.g. E- and I-cells recorded in all maps), grouped according to low-pass, broadband or high-pass characteristics in coherence plots. C) Distribution of cell classes across maps. The majority of cells in the CMS are low-pass, with a small number of broadband cells. Cells in the CLS are more equally distributed between low- and broadband populations. In the LS, all three classes of frequency tuning are observed, with low-pass cells making up approximately half of the cells.
remaining cells were either broadband or high-pass (Figure 6-6C). This suggested to us that the E-cells of the CMS and LS, consistent with their involvement with low-frequency and high-frequency behaviours, respectively (Metzner et al. 1998; Shumway 1989a), display specific frequency tuning due to the intrinsic conductances that shape firing dynamics. In comparison, E-cells of the CLS appear to be intrinsically broadband, which may contribute to the ability of feedback input to rapidly transform their frequency tuning into either high-pass or low-pass under specific in vivo stimulus conditions (Bastian et al. 2004; Chacron et al. 2003). The different frequency preference across cell types and maps may partially account for the observation that, when using 2-40 Hz RAMs in vivo, both E-cells as a population, and the population of LS cells show greater errors in feature detection in comparison to the population of I-cells, or of CMS cells respectively (Metzner et al. 1998). Specifically, this frequency range would be expected to reveal the largest differences across maps and between cell classes.

6.3.6 Spike characteristics correlate with frequency tuning

The heterogeneity observed in the frequency tuning of E-cells within maps suggested that frequency tuning may be a more important characteristic to correlate with the spike waveform characteristics, rather than the map within which they are located. We therefore compared the spike parameters calculated previously (AHP, threshold, and spike half-width), but grouped the cells by their frequency tuning characteristics rather than by their map of origin. By doing so we found that spike parameters vary most consistently with frequency tuning of the cell, rather than across maps. Low-pass cells
Figure 6-7 Spike characteristics of ELL pyramidal cells covary with frequency tuning.

The mean values of spike parameters for all E- and I-cells in all segments are shown when grouped according to the preferred frequency selectivity. (A) Spike half-width exhibits a significant increase with higher frequency tuning. (B) AHP size increases as the cell selects for higher frequencies. (C) Spike threshold increases in cells with higher frequency preferences. Sample sizes for recordings in each segment used to calculate histograms in are shown in brackets in (C).
showed the shortest spike half-width of any cell class (0.617 ± 0.019 ms), followed by broadband cells (0.73 ± 0.023 ms), with the widest half-widths in high-pass cells (0.98 ± 0.057 ms) (Figure 6-7A) (all populations were significantly different from all others, p < 0.05). We were able to find similar differences in the AHP, where cells belonging to all three categories of frequency tuning were significantly different from each other (low-pass: 6.06 ± 0.26 mV, broadband: 7.19 ± 0.44 mV, high-pass: 9.04 ± 0.60 mV, p < 0.05) (Figure 6-7B). The threshold for spike initiation in low-pass cells was significantly lower than in broadband or high-pass cells, which were not different from each other (low-pass: -67.0 ± 0.73 mV, broadband: -63.7 ± 0.86, high-pass: -60.6 ± 1.4 mV, p < 0.05) (Figure 6-7C). We conclude from this that although spike parameters rarely change significantly across maps when cells are grouped purely by map or cell class (see Figure 6-4), they do co-vary with frequency tuning. (CLS: 270.51 ± 20 Hz/nA, LS: 186.91 ± 17 Hz/nA, p > 0.05) (Figure 6-8A). These distinctions were further accentuated when the cells were grouped by their frequency-tuning characteristics to compare gain. When grouped by frequency tuning all cell classes were significantly different from each other, with the high-pass cells having the lowest gain (high-pass: 166.9 ± 22 Hz/nA, broadband: 316.3 ± 26 Hz/nA, low-pass: 405.33 ± 21 Hz/nA, p < 0.05) (Figure 6-8B). These data suggest that one of the outcomes of the differences is spike waveform across different frequency tunings is a variation of gain. This can be readily understood if it is considered that fast firing requires low frequency inputs, as high frequency time-varying inputs regulate the firing rate independent of the refractory period. In contrast, the firing rate in response to low-frequency inputs depends on the intrinsic refractory period. Thus higher gains encourage low-frequency responses, while a cell with lower gain would be expected to
Figure 6-8 Response gain changes with segment, cell type and frequency selectivity.

(A) Gain for I- and E-cells of different ELL segments. I-cells have a homogenous gain across segments, with no significant difference to CMS E-cells (including band-pass cells). In comparison, E-cells of the CLS and LS have significantly lower gain. (B) When classified by frequency tuning, the differences become more apparent: gain decreases significantly with higher frequency tuning characteristics. Sample sizes for recordings used to calculate means are shown in brackets.
fire fewer spikes in response to low-frequency stimulus components. The lower gain should not affect the response to higher frequency inputs, as under these conditions the limiting factor for the ISIs might be the statistics of the RAM and not the refractory dynamics of the cell.

### 6.3.7 Adaptation

Another factor which can contribute to frequency tuning is adaptation (Benda and Herz 2003; Benda et al. 2005). We therefore examined the distribution of adaptation rates across cells types and maps. Adaptation was characterized in response to the 0-60 Hz RAM stimulus (Figure 6-9A) and was well fit by two time constants. The first and largest of the time constants was invariably a fast decay between 0.5 and 4 s (representative data and fit is shown in Figure 6-9A,B). The second time constant was far more variable, and could consist of either a much smaller, slower decay, or display a gradual acceleration. This slow acceleration may be due to the persistent sodium currents previously observed to cause a slow acceleration in these cells (Berman et al. 2001; Doiron et al. 2003b). This small magnitude slow adaptation or acceleration could appear in any cell class, and could not be consistently related to any of our classifications. We focus primarily on the larger magnitude, fast component of adaptation, as these varied more reliably with cell class and across different maps.

We found that, consistent with many of the other spike characteristics observed here, a homogenous group was apparent in I-cells of the CMS (\(\tau = 2.69 \pm 0.24\) s), CLS (\(\tau = 2.48 \pm 0.30\) s), and LS (\(\tau = 2.36 \pm 0.10\) s), and CMS E-cells (\(\tau = 2.49 \pm 0.38\) s, \(p <\)
Figure 6-9 The rate of spike adaptation covaries across segments with frequency tuning.

A) Representative trace of the spike response of a pyramidal cell to a 0-60 Hz 100 s RAM intracellular current injection. B) Adaptation of spike rate in response to the RAM for the example shown in (A). Black lines show the instantaneous firing rate averaged in 1s bins. The grey line is the fit to the data. C) Distribution of adaptation time constants across ELL cell types and segments. E-cells of the CLS and LS have significantly shorter time constants for adaptation than other cell types in other segments. D) Adaptation time constant correlates with frequency selectivity characteristics, with a progressive and significant decrease in adaptation time constant with higher frequency tuning. Sample sizes for recordings used to calculate means are shown in brackets.
All of these cell types were significantly slower to adapt than E-cells of the CLS ($\tau = 1.46 \pm 0.17$ s) and LS ($\tau = 1.06 \pm 0.09$ s, $p < 0.05$) (Fig. 6-9B). When cells were instead grouped by their frequency-tuning characteristics, all three groups were significantly different from each other (low-pass: $2.54 \pm 0.17$ s, broadband: $1.73 \pm 0.04$ s, high-pass $1.06 \pm 0.04$ s, $p < 0.05$) (Figure 6-9C,D). We conclude that differences in adaptation are again among the factors which underlie the variability of frequency tuning across ELL maps.

6.4 Specific Discussion

We have used an in vitro slice preparation to examine the intrinsic cellular properties of two ELL pyramidal cell classes (E- and I-cells) across the three ELL maps receiving tuberous electroscopic input (CMS, CLS, LS). The primary findings are that:
(a) the properties of I-cells are relatively homogenous with no significant differences across the maps for any of the properties examined, including frequency tuning; (b) E-cells are highly variable across the maps with respect to many of the biophysical parameters examined including frequency tuning; (c) the biophysical differences between E-cells are strongly correlated with their intrinsic frequency tuning. In vivo recordings from ELL have produced concordant results with respect to frequency tuning. I-cells are generally low-pass in all the tuberous maps and frequency tuning of E-cells goes from low-pass (CMS) to high-pass (LS) (Shumway, 1989; Chacron et al. 2005, Krahe et al. pers. comm.). Note that these results were obtained under in vivo conditions where many factors, including feedback from higher brain centers, regulate frequency tuning. By
examining frequency tuning in the *in vitro* preparation we show that the differences in intrinsic channel properties or densities across E-cells may therefore account for at least part of the differences in frequency tuning within and across the ELL maps *in vivo*.

### 6.4.1 Classification of E- and I-cells by frequency tuning

Although in the CMS using frequency tuning to determine the morphological class of the pyramidal cells could lead to misclassification, any such errors would lead to the misclassification of superficial E-cells as I-cells. Because superficial E-cells have a low-pass response, this would lead to an increase in the number of low-pass cells classified as I-cells, and corresponding decrease in the number identified as E-cells. Any such misclassification would therefore decrease the variance in either group, and thus increase the likelihood of finding significant differences between CMS E- and CMS I-cells. As no such differences were found, we are confident in our conclusion that although E- and I-cells show differences they are, as a population, indistinguishable in the CMS.

### 6.4.2 Differences across cells, maps, and frequency tuning.

Different maps optimized to analyze distinct types of information are a commonly observed architecture for sensory processing (Metzner 1999; Schreiner and Winer 2007; Young 1998). In the auditory brainstem for example, repeating maps show specialization in terms of frequency, and are correlated with specific patterns of channel expression (Brew and Forsythe 2005; Li et al. 2001; von Hehn et al. 2004). In our examination of
ELL pyramidal cells, spike characteristics were most clear when cells were grouped by frequency tuning rather than by cell type or map, although such frequency tuning was dependent on both map and cell type. This was largely due to the heterogeneity of LS E-cell responses, which could be broadband or high-pass. In particular, E-cells appear to be the most variable across maps, and therefore should be examined specifically when candidate currents contributing to frequency selectivity are considered. We have confirmed that this distribution is the case for SK2 channels, which increase their expression in a medio-lateral fashion and contribute to the larger magnitude AHP in E-cells (Ellis et al. 2007b). Indirect evidence suggests that the high-threshold Kv3.1 channel might also be related to differences across maps. Expression of Kv3.1 increases from CMS to LS (Deng et al. 2005) and a high-threshold K⁺ channel (possibly Kv3.1) has been shown to stabilize high-frequency firing and maintain a brief spike-width in ELL pyramidal cells (Fernandez et al. 2005a). This suggests that in contrast to SK currents, high threshold K⁺ currents may be preferentially expressed in I-cells, as these cells have a narrow spike half-width and, in comparison to E-cells, are capable of higher frequency firing within each map. Further, these differences between E- and I-cells increase in the more lateral maps, suggesting that an increased Kv3.1 conductance may be contributing to some of the observed differences between E- and I-cells across the maps.

Our results suggest that the conductances underlying the observed differences in (a) spike waveform parameters (e.g. spike half-width, threshold and AHP), (b) spike threshold and gain, and (c) factors that control pyramidal cell firing patterns (e.g. adaptation and bursting) might all contribute to the frequency selectivity displayed by E-cells.
6.4.3 Spike half-width, AHP magnitude and frequency tuning

Spike half-width and the AHP magnitude co-varied with frequency tuning in a map-dependent fashion. Both features are likely associated with an increased magnitude and/or duration of the refractory state and might be expected to contribute to frequency tuning, as predicted by a theoretical analysis (Benda and Herz 2003). The refractory variable depends on both Na\(^+\) channel inactivation, and K\(^+\) channel activation. In fact, Na\(^+\) channel inactivation and K\(^+\) channel activation are often grouped into a single refractory variable for simplicity of analysis in some neural models, including this preparation (Fernandez et al. 2005b; Rinzel 1985). Alternatively, it may be due to K\(^+\) currents that contribute to the AHP, such as the SK2 (Ellis et al. 2007b) and/or Kv3.1 channel (Fernandez et al. 2005a; Noonan et al. 2003).

6.4.4 Spike threshold, gain and frequency tuning

Spike threshold and gain may initially appear to have little to do with frequency tuning, although high-frequency tuned LS E-cells do have an increased spike threshold compared to E-cells of CMS and CLS (Fig. 8C). In this regard, a recent computational analysis has shown that spike threshold, in combination with purely anatomical map differences (receptive field size), can contribute to frequency tuning (J. Middleton, pers. communication). The receptive field size of E-cells increases from CMS to LS (Shumway, 1989 for *E. virescens*; L. Maler, unpublished observation for *A. leptorhynchus*) and therefore many more P-units converge onto E-cells of the LS
compared to the CMS (the CLS is intermediate in this respect). A higher threshold in LS E-cells implies that they require synchronous input from many P-units to reach threshold (J. Middleton, pers. comm.). Since P-unit discharge is synchronized by high-frequency signals (Benda et al. 2006 and J. Middleton, pers. comm.), this further implies that high threshold pyramidal cells should be tuned to high-frequency input. The channels responsible for the differences in spike threshold are not known but one possibility is a differential expression of Na\(^+\) channels or associated subunits, which, through a shift in either inactivation or activation rates could both raise threshold and increase spike width. Another candidate is the persistent Na\(^+\) current, since this current is prominent in ELL pyramidal cells and enhances excitatory inputs and spike responses (Berman et al. 2001; Doiron et al. 2003b). Direct evidence for differential expression of persistent Na\(^+\) current in E-cells across maps is, however, lacking.

The effect of spike threshold is likely accentuated by the differential gain of E-cells across the maps. The mechanisms that underlie gain determine the ISI in response to the input, rather than the timing of individual spikes. Therefore high gains are only meaningful for inputs slow enough to generate two or more spikes. A low gain would be expected to decrease the response to low-frequency inputs since fewer spikes would be generated in response to the signal upstrokes. However, the lower gain should not affect the response to higher frequency inputs, as faster oscillations generally create single spikes for each stimulus upstroke (Oswald et al. 2004; Oswald et al. 2007). Under these circumstances the ISI is largely determined by the statistical properties of the time-varying input signal, rather than by the gain dynamics of the cell. The biophysical properties determining the intrinsic gain are not completely known but both subthreshold
Na\(^+\) and spike-initiated Na\(^+\) and K\(^+\) channels are likely to contribute (Fernandez et al. 2005b; Noonan et al. 2003).

We have previously shown that the intrinsic gain of pyramidal cells can also be regulated at the network level: dendritic inhibition caused by a specific interneuron- the VML cell- induces divisive gain control by reducing the somatic DAP emanating from pyramidal cell apical dendrites (Mehaffey et al. 2005) (Chapter 3). The VML cell receives its input entirely from feedback to the ELL (Maler 1979; Maler and Mugnaini 1994). It is therefore interesting that the LS has the highest density of VML cells (Maler 1979; Maler and Mugnaini 1994; Shumway 1989a). This suggests that, more generally, feedback control of gain (Bastian 1986a; b; Mehaffey et al. 2005) and frequency tuning (Bastian et al. 2004; Chacron et al. 2003) might be linked through synaptic regulation of the channels responsible for the differential expression of gain across the ELL tuberous maps.

6.4.5 Spike frequency adaptation and frequency tuning

As discussed by Benda and Herz (2003) temporal summation of adapting currents contributes to spike frequency adaptation and, as discussed above, this promotes high frequency tuning. We have confirmed that E-cells tuned to higher frequencies show faster adaptation (Figure 6-9). We observed that these neurons are also those least likely to generate bursts, both in response to DC steps and to time-varying inputs. This faster rate of adaptation is expected to be recruited rapidly during slow stimulus upstrokes, which result in faster ISIs than are generated by the higher frequency components of the
stimulus. This fast firing will recruit adapting currents and therefore downregulate the response to low-frequency stimulus components. However, adaptation is unlikely to accumulate as strongly in response to fast stimulus components which generate primarily single spikes, permitting a more rapid recovery during stimulus-driven pauses in spiking, and in turn allowing high-frequency components to be coded more accurately. The biophysical basis of spike-frequency adaptation in pyramidal cells is not currently known, but blockade of SK currents with apamin does not prevent adaptation in E-cells (WHM, unpublished observation), suggesting that another Ca\(^{2+}\)-activated K\(^+\) current might be involved. This is consistent with observations in the lateral amygdala where SK currents do not contribute to spike-frequency adaptation (Faber and Sah 2002).

6.4.6 Bursting and low frequency tuning

Many studies have now shown links between burst discharge and low frequency events in the electrosensory system (Doiron et al. 2007; Krahe and Gabbiani 2004) and in other sensory systems (Krahe and Gabbiani 2004; Lesica and Stanley 2004; Lesica et al. 2006). This appears to be a useful adaptation for generating distinct spike patterns in response to specific features of a sensory signal. In our examination of intrinsic bursting we find that the distribution of such bursting cells varies, occurring most frequently across maps in association with cells that prefer low-frequency events. This included I-cells in all three maps. We found that bursting was less commonly observed in E-cells than I-cells, particularly in more lateral maps (Figure 6-1D). Further, E-cells show lower stimulus-response coherence at low frequency (0-20 Hz), particularly in the CLS and LS.
This is consistent with our previous observations that conductances that can regulate burst threshold are able to decrease the low-frequency component of the stimulus-response coherence (Ellis et al. 2007b). In a recent paper, we examined the contribution of an SK-mediated AHP to frequency tuning in response to broadband inputs (Ellis et al. 2007b). This current may make an important contribution to the relationship between AHP and frequency selectivity observed here, but it is interesting to note that although apamin increased the low-frequency coherence in broadband or high-pass cells, it did not alter their high-pass characteristics. This suggests that the SK2 current downplays the low-frequency coherence, rather than amplifying the high-frequency coherence. Therefore at least one other conductance is likely required to confer high-pass tuning. One candidate current would be the A-type K⁺ channels which have been described in ELL pyramidal cells (Ellis et al. 2007a; Mathieson and Maler 1988) and that can decrease a neuron’s response to low-frequency input (Ellis et al. 2007a). Further, the A-type channel is modulated by muscarinic acetylcholine receptors and this in turn regulates their frequency tuning (Ellis et al. 2007a). This suggests that synaptic inputs can modulate pyramidal cell frequency tuning via second messenger regulation of intrinsic conductances as well as directly via voltage-gated channels (see above).

6.4.7 I-cells and low frequency tuning

We found that I-cells exhibited low frequency tuning across all three tuberous maps. The low-frequency tuning observed in vivo (Chacron et al. 2005; Krahe et al. 2008) is therefore due, at least in part, to the intrinsic properties of I-cell, such as their
greater propensity to burst as well as their narrow spike width and higher gain, allowing a
greater response to low frequency stimulus components. The intrinsic low-frequency
tuning of I-cells might be accentuated by the neuronal architecture of the ELL: since I-
cells receive inputs indirectly through the granular cell interneurons, it is possible that
this disynaptic relay may contribute to low-pass tuning. It should also be noted that
decreases in the EOD amplitude (e.g. the stimuli for which I-cells are selective) typically
result from the fish swimming past non-conductive objects and that this generates large,
low-frequency signals, relative to the small amplitude increases in EOD amplitude at the
tailing edges of the electric image (Chen et al. 2005; MacIver et al. 2001). Thus the
intrinsic and network-dependent tuning properties of I-cells may be well suited for the
detection of inanimate non-conductive environmental stimuli such as rocks, although
other contexts may lead to large amplitude decreases in EOD (e.g. AM beats between
conspecifics which can contain both high and low frequency components).

The vital role of frequency tuning in the ELL is clearly illustrated by the observed
ccontributions at every level of neuronal processing. From the high-frequency selectivity
of synchronized pre-synaptic activity (Benda et al. 2006)(J. Middleton, personal
communication) to the regulation of conductances that underlie spike generation and
firing patterns (Ellis et al. 2007a; Ellis et al. 2007b; Fernandez et al. 2005a; Turner et al.
1994), to feedback regulation of frequency tuning by higher brain centers (Chacron et al.
2003; Chacron et al. 2005), many factors contribute to the computations performed by
ELL pyramidal cells. As we show here, the spike generating mechanism is a key factor
underlying the potential for further regulation of frequency tuning by feedback or
feedforward networks.
Chapter Seven: Discussion

This thesis has examined several aspects of the dendritic regulation of somatic excitability. Inarguably, the soma and axon are the key regions of spike initiation and for the propagation of action potentials throughout the nervous system, an integral component of any neural computation. The dendrites, however, can also generate regenerative events which can create a DAP at the soma which can influence the final spike output of pyramidal cells. A major component of my work presented here is to delineate how this simple soma-dendritic interaction can control cell output, and in turn how it is regulated by inhibitory synaptic feedback in relation to functionally relevant behavioural conditions. As shown in Chapter 3, this form of dendritic feedback underlies an entirely deterministic mechanism of gain control, and one that is regulated by inhibitory GABAergic synaptic inputs in a subtractive or divisive manner depending on the site of synaptic termination (Chapter 3). I further examined a distinct GABA_B-mediated mechanism by which this feedback regulation can increase burst excitability. This mechanism occurs through a selective regulation of dendritic spike latency, and the resulting delay in DAP timing interacts with somatic firing dynamics. We extended this analysis to include a small network to further test how feedback pathways and their specific sites for synaptic termination could improve sensory coding (Chapter 5). A further examination of this and other spike properties established how the intrinsic firing properties of pyramidal cells is regulated according to cell subtype and across topographical maps to establish differential frequency tuning (Chapter 6). As my work has shown, the dendrite can play a significant role in regulating all of these firing
dynamics. I expand on the key results that support each of these novel aspects of dendritic control of excitability below.

7.1 Mechanism of burst firing in ELL pyramidal cells

It has been well established that ELL pyramidal cells display a characteristic form of bursting, and that this bursting is driven by a shift in the relative influence of the dendritic compartment on somatic excitability (Fernandez et al. 2005a; Lemon and Turner 2000; Turner et al. 1994). This change in the properties of active spike discharge in the dendritic compartment leads to a depolarized dendrite that overlaps in time the period of spike repolarization at the soma. The resulting voltage difference causes a flow of current from the dendrite to the soma, increasing somatic excitability. This dendrosomatic voltage difference leads to the generation of a DAP, which drives bursting in ELL pyramidal cells. This bursting, at least phenomenologically, is conserved across many commonly studied species of Gymnotiform fish, including *Apteronotus leptorhynchus*, *Apteronotus albifrons*, and *Eigenmannia viriscens* (Mehaffey et al. 2008a). Through collaborative work, we showed that this process actually reflects a progressive change in the temporal relationship between somatic and dendritic spikes, with a progressive increase in dendritic spike (and thus DAP) latency (Fernandez et al. 2005a; Lemon and Turner 2000; Turner et al. 1994). This process proved to be driven primarily by a gradual inactivation of dendritic Na\(^+\) channels during high frequency repetitive discharge (Fernandez et al. 2005a). Paradoxically, this decrease in dendritic excitability is able to drive an increase in somatic excitability, as reflected in both a
decrease in ISI and shift to burst output through a change in the efficacy of the DAP. My work has now expanded considerably on this to further understand how the DAP controls gain, and how the DAP is in turn controlled by feedback inhibition to control sensory processing by pyramidal cells.

7.2 Gain control by regulation of the DAP

A number of studies have determined possible mechanisms to control the gain of an individual cell. Dynamic clamp (Chance et al. 2002; Mitchell and Silver 2003) and modelling of various configurations of synaptic input (Burkitt et al. 2003; Doiron et al. 2001a; Longtin et al. 2002; Murphy and Miller 2003; Prescott and De Koninck 2003; Salinas and Sejnowski 2000; Tiesinga et al. 2000) have shown how noise is capable of generating divisive effects. These studies were able to modulate FI curves such that they maintained a stationary input threshold for initial spiking (rheobase), while the gain of cell output (in Hz/nA) decreased with both increasing inhibition or with balanced excitation and inhibition. This gave an overall division of the averaged firing rate relative to the injected current. However, such a noise-induced gain mechanism requires that the membrane fluctuations drive spiking in a probabilistic fashion, suggesting that as the window of measurement decreases, the likelihood of successfully encoding inputs will be reduced, preventing the faithful encoding of brief transients.

We report in Chapter 3 that in the ELL, a prominent result of the DAP is an approximately multiplicative increase in gain. Moreover, this system allows inhibitory input to regulate gain in a purely deterministic fashion without the deleterious effects of
noise. It has been shown that the inhibitory VML cells specifically project GABAergic inputs to the region of pyramidal cell apical dendrites involved in generating the DAP (Maler and Mugnaini 1994; Turner et al. 1994). The specific spatial pattern of VML cell inhibition thus allows these cells to modify gain in a deterministic fashion by regulating the influence of the DAP. I first established that regulation of the dendritic spike waveform by inhibition with appropriate spatial distribution of the synapses is able to dilute the multiplicative effect of the dendritic depolarization, reducing pyramidal cell gain in a deterministic fashion. This in turn allows for a division of the instantaneous firing rate, rather than a division of the averaged firing rate.

7.3 Functions of somatic and dendritic GABA receptor activation

The effects of inhibition on ELL pyramidal cell spike output are complex. For instance, as described in Chapter 3, GABA_A inhibition can cause subtractive or divisive effects on cell output depending on the location of the synapses (Mehaffey et al. 2005). In contrast, the proximal apical dendritic GABA_B receptors (but not GABA_A) can selectively regulate burst dynamics in relation to activity in a specific feedback pathway. It is thus important to consider not only the site of synaptic termination but also the subtype and time scale of inhibition, as well as the cell’s intrinsic firing dynamics. In this case, the manipulation of the firing dynamics is accomplished through regulation of dendrosomatic feedback through a DAP. Recent work has elucidated the role of the DAP in creating specified burst ISI which allows coding for the amplitude of low frequency events (Oswald et al. 2007), and theoretical work has suggested that regulation of the
DAP has the potential to modulate this code (Doiron et al. 2007).

Differential impacts of somatic and dendritic inhibition on the electrophysiological (e.g. spiking) properties of neurons have been reported previously (Mehaffey et al. 2005; Vu and Krasne 1992) and may allow different sets of inhibitory interneurons to selectively regulate patterns of spike generation. The activation of GABA_A and GABA_B receptors on specific subregions of the dendrite cause distinct effects. GABA_A receptor activation only reduced the late phase of the dendritic spike, while GABA_B receptor activation increased the relative onset latency of the dendritic spike by regulating the time for the dendritic spike to cross threshold. The apparent lack of effect by GABA_B receptors on the late phase of the dendritic spike may simply reflect the more active repolarization of spikes in the proximal dendritic regions. In contrast, the more distal dendritic GABA_A (~100 μm) conductances regulate the shape of the backpropagating spike with less of an effect on rheobase or firing dynamics. GABA_B inhibition may be unable to sufficiently regulate the narrower dendritic spike waveform observed in more proximal regions of the dendrite, preventing the decrease in gain seen with GABA_A agonists.

7.4 Synaptic regulation of backpropagating dendritic spikes

We summarize these results in the context of ELL anatomy in Figure 7-1. GABA_A inhibition can regulate excitability of ELL pyramidal cells in distinct fashions based on the site of inhibition. A backpropagating dendritic spike, and hence the DAP, can be regulated by proximal dendritic inhibition mediated by the VML cell (Figure 7-1
green), causing a divisive modulation of the gain. In contrast, somatic inhibition through the feedforward GC2 inhibitory interneurons subtractively modulates the FI relationship (Figure 7-1, black). Thus the inhibitory current mediated by GABA_A receptors can have dramatically different effects depending upon their spatial localization and the presynaptic cell of origin. In contrast, GABA_B receptor mediated currents generated by descending feedback inputs from nP bipolar cells can regulate burst dynamics by increasing the temporal separation between somatic and dendritic spikes (Figure 7-1, blue). This can in turn tune the spiking response of ELL pyramidal cells to complex inputs, allowing a sharper distinction between bursts and isolated spikes involved in coding specific features of different behaviourally relevant inputs.

One further network effect predicted by the anatomy of the ELL (Maler and Mugnaini 1994) involves the inhibition of VML cells (Berman and Maler 1998a). Previous anatomical studies showed that nP bipolar cells can provide inhibitory input to VML cell somata (Maler and Mugnaini, 1994). Since VML cell mediated inhibition reduces the gain of pyramidal cells (Mehaffey et al. 2005), inhibition of this interneuron should, in turn, increase the gain of pyramidal cells. We have not yet examined the possible interactions in detail, and these will depend crucially on the kinetics and
Figure 7-1 Summary of feedforward and feedback pathways influencing spiking in ELL pyramidal cells.

GABA\textsubscript{A} inhibition can regulate excitability of ELL pyramidal cells in distinct fashions based on the site of inhibition. Divisive modulation of gain can be accomplished by regulating the backpropagating dendritic spike. This is due to proximal dendritic GABA\textsubscript{A} receptor mediated inhibition by projections of the VML cell (green). Somatic inhibition through the feedforward GC2 inhibitory interneurons instead modulates gain in a subtractive fashion (black). In contrast, GABA\textsubscript{B} receptor mediated currents activated by feedback from bipolar cells of the nP can regulate burst dynamics by modifying the temporal relationship between somatic and dendritic spike discharge (blue). The interaction(s) between these pathways remains to be examined, but the anatomy shows that the VML cell also receives bipolar cell inhibition, suggesting a further means by which GABAergic inputs could modulate pyramidal cell burst output.
subtypes of inhibitory receptors on the VML cell. Little is known of the in vivo firing behaviour of VML cells, although in vitro they can fire rapidly (> 200 Hz, WH Mehaffey, unpublished observation). The synaptic dynamics will also play an important role, both for the VML cell, and for the parallel fibres that provide much of the net excitatory feedback to pyramidal cell dendrites in the DML. Parallel fibre to ELL pyramidal cell synapses show complex dynamics (Lewis and Maler 2002; 2004), which may also influence VML behaviour. In contrast, nP bipolar cells are known to be preferentially recruited by appropriate, spatially coherent input (Doiron et al. 2003a). The complexity of synaptic interactions with intrinsic firing dynamics can only increase as one takes into account short term synaptic dynamics (Lewis and Maler 2002; 2004), feedback delays (Doiron et al. 2003a; Doiron et al. 2004; Lindner et al. 2005), receptor subtype, and spatial distribution (Mehaffey et al. 2005; Mehaffey et al. 2007).

7.5 Dendritic influence on spikes and burst coding

In response to broadband dynamic inputs, ELL pyramidal cell bursts obtained both in vivo and in vitro rarely involve more than two spikes (Gabianni et al., 1996; Oswald et al., 2004; Oswald et al., 2007). This is qualitatively distinct from the multi-spike burst dynamics originally identified by injection of static depolarizing current in vitro (c.f. Figure 1-4). It has recently been shown that one reason for this is that broadband dynamic stimuli may interrupt the slow dynamics of the burst, allowing only static or slow inputs (~ 10 Hz and less) to elicit the full response characterized in vitro
(Doiron et al., 2007). This range of optimal frequencies at which the full slow dynamic occur suggests that bursts may be an important response to low frequencies, including the JAR, environmental, and prey-like electrosensory stimuli. Interestingly, some recent results suggest that bursting is common in the CMS, which is known to be vital for the JAR (Metzner and Juranek 1997), but less common in the LS (Mehaffey et al. 2008b) which contributes to communication behaviours (Metzner and Juranek 1997). Nevertheless, modelling results show that the influence of the DAP on spike discharge remains critical to account for the burst response to fast transients as well (Doiron et al., 2007). We demonstrate in Chapter 4 that GABA_B mechanisms that manipulate the burst response in static input scenarios (c.f. Figure 4-6, Figure 4-7) retain their influence on the burst response to dynamic inputs. This is due to the synaptic inputs influencing the shape and timing of an individual dendritic spike, and hence the DAP, rather than the slow dynamics created by the effective potentiation of the DAP by a gradual shift of DAP timing. Thus, while the full slow burst response to static inputs may be unique to ELL pyramidal neurons, the response of these cells to dynamic inputs and the regulation of this response by selective inhibition to dendrites may be reveal a general coding strategy of a wide variety of neurons in the CNS, requisite only on the generation of dendro-somatic excitatory feedback.

7.6 Network mediated oscillations and burst regulation

We also examined the regulation of bursting in response to a more dynamic input. We initially examined synaptic regulation of burst output using relatively static inputs,
either by application of pharmacological activators or by modelling the activity induced by a tonic current (Chapter 5). It is however, known that these inputs are not tonic, but are recruited dynamically as part of a well understood feedback loop. Since testing this interaction is experimentally intractable at this time, we chose to use a computational approach (Chapter 5). Using parameters from an earlier model of this neuronal feedback loop (Doiron et al. 2003a), we incorporated our two-compartment model of bursting and burst regulation (Fernandez et al. 2005b; Mehaffey et al. 2007) into a model of the ELL-nP feedback loop. We then were able to show that many of our initial results observed under conditions of tonic application of a GABA\textsubscript{B} receptor agonist were well conserved even in a more realistic, dynamic context. Further, we found that the network mediated oscillation and regulation of burst dynamics were complementary, and could in fact aid the segregation of stimulus-specific frequency components into distinct components of the spike train.

### 7.7 Relation of bursts to sensory coding

Spikes have been assumed to be the main channel for information transfer between neurons (Eggermont 1998; Rieke 1997) although the roles for spike bursts or other patterned activity in signal coding have begun to be studied, particularly in sensory systems (Krahe and Gabbiani 2004). A segregation of spike trains into bursts and single spikes by sensory neurons has been observed in electric fish (Gabbiani et al. 1996; Oswald et al. 2004) and in the mammalian visual (Lesica and Stanley 2004; Lesica et al. 2006) and auditory systems (Eggermont and Smith 1996). Remarkably, bursts but not
isolated spikes, even predict behaviour in an invertebrate preparation (Marsat and Pollack 2006). Bursts have been suggested to code for input slope (Kepecs et al. 2002) and to segregate different components of stimuli (Doiron et al. 2007; Kepecs and Lisman 2003; Oswald et al. 2007). In sensory systems, bursts have been shown to be the preferred response for low frequency events (Lesica and Stanley 2004; Lesica et al. 2006), and specifically in the ELL for detecting low frequency features of a stimulus (Doiron et al. 2007; Gabbiani et al. 1996; Metzner et al. 1998; Oswald et al. 2004; Oswald et al. 2007). As such, bursts appears to be an extremely common adaptation with clear benefits to information coding. When information is parceled into two distinct ranges of ISIs, the system can take advantage of differences in the postsynaptic threshold for spiking, short term synaptic plasticity, or the intrinsic dynamics of downstream cells in order to separate and decode information. Such concepts have been analyzed in more detail elsewhere - in particular that bursts tend to be more reliably timed, show superior feature detection properties, and may be able to more reliably activate downstream cells than single spikes (Gabbiani et al. 1996; Izhikevich et al. 2003; Kepecs and Lisman 2003; Lisman 1997; Metzner et al. 1998).

It has recently been suggested that the slow transition in burst dynamics that can be found in pyramidal cells during in vitro recordings (i.e. 3-5 spikes per burst) is important when sensory input is comprised of the low frequencies characteristic of local prey stimuli (Doiron et al. 2007). In the presence of broadband inputs containing both high and low frequency signals, ELL pyramidal cells instead respond predominantly with doublets rather than full bursts (Gabbiani et al. 1996; Metzner et al. 1998; Oswald et al. 2004; Oswald et al. 2007). Thus, a compression of the tonic firing region resulting in
doublet spike discharge (as we observed upon GABA_B receptor activation) is consistent with the typical response to broadband inputs *in vivo*. The importance of the full burst dynamics for the processing of signals consisting primarily of low frequency inputs remains to be more thoroughly examined, and will require a study of the synaptic and intrinsic dynamics of midbrain target neurons.

### 7.8 Decoding of bursts and spikes

Any encoding scheme is obviously without meaning if it cannot be decoded. To consider how ELL pyramidal cell firing may be decoded, we consider the neurons immediately upstream. ELL pyramidal cells project to both the nP and to the torus semicircularis (TS), the two second order structures in electro sensory processing. The nP is capable of feeding back to the ELL either directly or indirectly, while the TS projects to higher order nuclei.

It has been established that the lemniscal afferents driving cells in the TS show a rapidly recovering form of short term depression (Fortune and Rose 2001; 2000), suggesting that later spikes in a fast burst will be weighed less heavily than initial spikes. Such a mechanism might be capable of coding preferentially for the longer ISIs of isolated spikes. Further, such isolated spikes fire coincidentally regardless of social context cues (Chacron and Bastian 2008). Taken together, this suggests that toral cells may receive stronger excitation via synchronous generation of isolated spikes across a population of ELL pyramidal cells, but that sustained bursting would weaken synapses and decrease excitation.
In comparison, the nPd stellate cells may be well suited to decode bursts. Recently, the dynamics of the ELL to nPd stellate cell synapse were examined, which showed little short term depression at high firing frequency (Mileva et al. 2008). The nP stellate cell provides direct excitatory feedback to the ELL (Bastian and Bratton 1990; Berman and Maler 1998a). Their minimal short term depression suggests that, unlike lemniscal afferents projecting to the TS, they do not appear to filter incoming inputs at the ISIs related to bursting. This suggests that bursts in ELL pyramidal cells may be able to drive nP stellate cells particularly well. Indeed, in vivo recordings show that stellate cells show their peak response to EOD AM frequencies near 10 Hz. This is the frequency range that has been shown to drive bursting in ELL pyramidal cells (Mehaffey et al. 2007; Oswald et al. 2004). In particular, this frequency range is particularly prevalent in stellate cells coding for downstrokes in EOD AMs (e.g. stellate I-cells), which receive inputs from pyramidal I-cells that also code for EOD downstrokes, which in turn show the greatest propensity to burst in response to time-varying inputs (Mehaffey et al. 2008b).

7.9 Dendritic regulation of somatic excitability

Backpropagating dendritic spikes have been shown to have many roles in determining cell output, including multiple forms of synaptic plasticity (Froemke et al. 2005; Gomez et al. 2005; Reyes 2001; Sawtell et al. 2007; Sjostrom and Hausser 2006). The work in the present thesis has served to highlight the significant role that dendrites can play in regulating spike output dynamics. Repetitive activation can change somatic
excitability through shifts in dendritic spike properties (Fernandez et al. 2005b), while
dendritic inhibition can have drastically different roles on somatic spike output depending
on their termination site and the receptor subtype activated (Mehaffey et al. 2005;
Mehaffey et al. 2007). Dendrosomatic feedback further underlie a novel, deterministic
form of gain control in ELL pyramidal cells (Mehaffey et al. 2005) that can be selectively
regulated by feedback inhibitory inputs (Mehaffey et al. 2005; Mehaffey et al. 2007).
Moreover, this system has allowed me to assess the potential functional significance of
soma-dendritic interactions in an ethologically relevant fashion using interactions
between a network model and the intrinsic cell dynamics to examine coding of
behaviourally relevant frequencies. The extent to which other cells in the CNS
incorporate this particular form of conditional backpropagation remains to be determined,
but the ubiquitous nature of dendritic spike backpropagation suggests that these forms of
soma-dendritic interactions have widespread potential to influence CNS function.
Appendix 1: INCLUSION OF WORK PUBLISHED OR SUBMITTED BY CANDIDATE


Chapter 4: Mehaffey WH, Fernandez FR, Maler L, and Turner RW. “Regulation of burst dynamics improves differential encoding of stimulus frequency by spike train segregation.”, J. Neurophysiol. 2007 Aug;98(2): 939-951. [WHM designed experiments, performed experiments, and modelling and analyzed data. FF designed experiments and created the original model used here]

Chapter 5: Mehaffey WH, and Turner RW. “Feedback Modulation of Firing Dynamics Restores Feature Detection in Electrosensory Processing”, In Preparation. [WHM built the model, designed experiments and analyzed data].

Chapter 6: Mehaffey WH, Maler L, and Turner RW. “Regulation of intrinsic frequency tuning of ELL pyramidal cells across electrosensory maps.” J. Neurophysiol. 2008. [WHM designed experiments, performed experiments and analyzed data].
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