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Burst Firing in Electrosensory Processing

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Burst Firing in Electrosensory Processing

Anne-Marie Michelle Oswald

This thesis is submitted as a partial fulfillment
of the Ph.D. program in Neuroscience

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Department of Cellular and Molecular Medicine
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Abstract

Processing of sensory input requires information transfer between neurons primarily via action potentials. The interaction between synaptic input and the ionic mechanisms of action potential generation leads to stereotyped sequences of action potential events. It is hypothesized that these spike sequences code for the sensory inputs. Spike trains often consist of sequences of single action potentials and clusters of action potentials or bursts. Investigations in this thesis are directed toward identifying candidate coding mechanisms in the processing of electrosensory input by pyramidal cells of the electrosensory lateral line lobe (ELL). These studies employ electrophysiological techniques combined with computational analysis and neural modeling to study the responses of pyramidal cells to mimics of electrosensory stimuli and feedback from higher brain centers. The major findings of this thesis are that the timing of isolated action potentials is correlated with a broad range of frequencies present in electrosensory stimuli whereas burst timing is correlated mainly with low frequency stimulus content. The timing of both single action potentials and bursts is reproducible and occurs with low variability in response to repeated stimulus presentations. In addition, within burst events, the time intervals between consecutive action potentials are correlated with stimulus intensity. Thus, bursts can detect low frequency stimulus events as well as estimate their intensity. Finally, feedback input to ELL pyramidal cells from higher brain regions is highly plastic. A subset of these feedback fibers synapse on the region of pyramidal cell dendrites that are responsible for burst generation; thus feedback synaptic input might modulate burst responses and perhaps enhance the detection of low frequency stimuli. The main conclusions of this thesis are that isolated action potentials and spike bursts code for

different stimulus frequencies and that the time between burst spikes codes for the intensity of stimulus events. In addition, I speculate that feedback can modify coding by regulating bursting.

Le traitement des entrées sensorielles nécessite un transfert d'information entre les neurones principalement au moyen de potentiels d'action. L'interaction entre les entrées synaptiques et les mécanismes de génération des potentiels d'action entraîne des séquences stéréotypées d'événements de potentiels d'action. On a émis l'hypothèse que ces séquences codent l'information des entrées sensorielles. Les trains de potentiels consistent souvent en combinaisons de potentiels d'action isolés et en paquets de potentiels d'action appelés rafales. Les études durant cette thèse sont dirigés vers l'identification de candidats codant les mécanismes du traitement des entrées électrosensorielles par les cellules pyramidales du lobe électrosensoriel de la ligne latérale (ELL). Ces recherches emploient une combinaison de techniques d'électrophysiologie, d'analyse computationnelle et de modélisation neuronale pour étudier la réponse des cellules pyramidales à reproduire les stimuli électrosensoriels et les rétroactions provenant des centres cérébraux supérieurs. Les principaux résultats de cette thèse montrent que les potentiels d'action isolés se produisent sur une large bande de fréquences contenues dans les stimuli sensoriels, tandis que les rafales de potentiels d'action se produisent suite à des stimuli de basses fréquences. Suite à une présentation répétée des stimuli, les patrons temporels des potentiels d'action isolés et des rafales sont très reproductibles et montrent une faible variabilité. De plus, à l'intérieur des rafales, il y a une corrélation entre les intervalles de temps des potentiels d'action consécutifs et l'intensité du stimulus. Ainsi, les rafales de potentiels d'action peuvent détecter les stimuli de basse fréquence de même que leur intensité. Finalement, les entrées synaptiques de rétroaction des cellules pyramidales du ELL provenant des centres cérébraux supérieurs sont hautement plastiques. Une portion de ces synapses de

rétroaction situées dans la région apicale des dendrites des cellules pyramidales sont responsables de la génération des rafales ; ainsi les entrées synaptiques de rétroaction peuvent moduler les réponses en rafale et peut-être augmenter la détection des stimuli de basses fréquences. Les principales conclusions de cette thèse sont que les potentiels d'action isolés et des rafales codent différentes fréquences du stimulus et que l'intervalle de temps des potentiels d'action durant les rafales code l'intensité des événements du stimulus. De plus, je propose que la rétroaction peut modifier la codification en régulant les mécanismes de rafales des potentiels d'action.

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List of Abbreviations

A	Adenosine
ACSF	Artificial cerebro-spinal fluid
ADM	Active dendrite model
ADP	After depolarization
AHP	After hyperpolarization
AM	Amplitude modulation
AMPA	γ -amino-3-hydroxy-5-methyl-4-isoazolepropionic acid
ANOVA	Analysis of variance
bAHP	Burst after hyperpolarization
BEV	Burst Event Fraction (number of burst events/ total events)
BF	Burst Fraction (number of spikes in bursts/ total spikes)
BTA	Burst triggered average
C	Cytosine
CAMKII	Calcium calmodulin kinase II
cf	Coding fraction
C_{high}	Area under the coherence curve between 40-60 Hz
C_{low}	Area under the coherence curve between 0-20 Hz
CLS	Centrolateral segment
CMS	Centromedial segment
D	Dorsal
d	Dendrite
D	Deep pyramidal cell
DAP	Depolarizing after potential
DFL	Deep fiber layer
D_i	Depression, $i=1$ or 2
DML	Dorsal molecular layer
DNA	deoxyribonucleic acid
DNL	Deep neuropil layer
EGp	Eminentia granularis posterior

ELL	Electrosensory lateral line lobe
EOD	Electric organ discharge
EPSP	Excitatory post-synaptic potential
E-type	Excitatory –type pyramidal cells (on-type)
f	Optimal feature
f_c	Cut off frequency
F/I	Frequency/Current
FD	Frequency-depression model
F_i	Facilitation, $i=1, 2$ or PTP
G	Guanine
GABA _A	Gamma-amino-butyric-acid receptor A-type
GCL	Granule cell layer
HF	High frequency
H-H	Hodgkin-Huxley
I	Intermediate pyramidal cell
IPSC	Inhibitory post synaptic current
IPSP	Inhibitory post synaptic potential
ISI	Inter-spike interval
I-type	Inhibitory–type pyramidal cells (off-type)
K _{V3.3}	Voltage dependent potassium channel-3.3
L	Lateral
LF	Low frequency
LGN	Lateral geniculate nucleus
LIF	Leaky-integrate and fire model
LS	Lateral segment
M	Medial
MS	Medial segment
MSP	Minimum sustained potentiation
NMDA	N-methyl-D-aspartate
PC	Pyramidal cell
PCL	Pyramidal cell layer

Pd	Nucleus praeminentialis
p_d	Slow dendritic variable
P_D	Probability of correct detection
PDM	Passive dendrite model
PF	Parallel fibers
P_{FA}	Probability of false alarm
P_{high}	Area under the power spectrum curve between 40-60 Hz
PIL	Plexiform layer
P_{low}	Area under the power spectrum curve between 0-20 Hz
PPF	Paired pulse facilitation
PTP	Post-tetanic potentiation
P-unit	Probability unit- amplitude coder
P_e	Probability of error
RAM	Random amplitude modulation
ROC	Receiver operating characteristic curve
s	Soma
S	Superficial pyramidal cell
st	Stellate cell
$s(t)$	Stimulus vector
SNR	Signal to noise ratio
STA	Spike triggered average
STDP	Spike timing dependent plasticity
Stf	Stratum Fibrosum
T	Thymine
TS	Torus semicircularis
TTX	Tetrodotoxin
T-unit	Time unit- phase coder
U	Uracil
V	Ventral
V_m	Membrane potential
VML	Ventral molecular layer

X_p	Activation
XPP	X-Windows Phase-Plane (simulation & analysis program)
Y_p	Deactivation

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Introduction

Overview

During sensory processing, peripheral receptors and neural circuits transform the events of the outside world into a series of action potentials. This encoded information is relayed from peripheral receptors through intermediary sorting and processing centers to the cortex which mediates our perceptions and initiates behaviours. One fundamental question in neuroscience research asks, “What is the neural code by which neurons transform and relay information?”

This thesis will address this question using the electrosensory system of the brown ghost knife fish, *Apteronotus leptorhynchus* as a model of sensory processing. The main hypothesis of this work is that burst firing is a salient feature in the coding of electrosensory stimuli. The ensuing chapters endeavor to answer the following questions with respect to burst firing and electrosensory processing. Which features of stimulus are encoded? How are these features represented in the code? How precisely and reliably is the information encoded? Can the information be quantified? Can information transfer be modulated?

The studies described in the present work are a combination of electrophysiological, computational and theoretic approaches to neuroscience research. The introduction will comprise of an overview of neural coding, the electrosensory system, the Hodgkin-Huxley models of action potential generation, a description of the mechanisms and models of electrosensory burst firing, the role burst firing in neural processing and signal detection theory.

Neural Coding

The concept of a neural code while seemingly straightforward is in practice difficult to define. From a cryptology standpoint, a code is a substitution scheme, or cipher, where letters or phrases in the message are replaced by a special set of symbols. If one has the key, the encoded information can be decoded and the exact version of the original text recovered. The genetic code is a biological example of this type of coding. Amino acid sequences are represented in DNA as nucleotide triplets of adenosine (A), guanine (G), thymine (T) and cytosine (C) that are copied (with a substitution of thymine to uracil (U)) and conveyed as messenger RNA to the cytoplasm. The cipher is decoded by transfer RNA which contains triplet “keys” that correspond to individual amino acids and the original intended sequence of amino acids is recovered.

In neural systems, an analogous description of coding is more complicated. It is generally accepted that information is coded in sequences of action potentials. Classically, neurons receive input at their dendrites or soma through chemical or electrical synaptic contacts; if the summed input reaches a threshold potential (about -50 mV) at the axon hillock, the information contained in the input is transmitted via action potentials to target cells. The existence of a threshold for action potential generation demonstrates that this may not be a simple one-to-one, input-output relationship. Action potential sequences are dependent not only on the timing and the quality of the inputs but also the state of the neuron. Neural responses are subject to intrinsic properties such as adaptation and oscillatory states as well as network interactions. Thus, the information encoded in a sequence of action potentials is not only a reflection of the information in the input but also of the state of the neuron when the input was received. For this reason

the identification of a single neural code or specific “key” to crack the code is unlikely. It is more plausible that a number of different codes exist, serving at different levels of information processing.

The present work is motivated by the definition of neural coding as stated by Perkel and Bullock (1968), “[Neural coding is the] representation and transformation of information in the nervous system”. These authors outline steps in identifying candidate neural codes and a number of recent reviews of potential codes have generally followed these guidelines (Theunissen, 1995; Borst, 1999; Lestienne, 2001). The first step is the identification of the *referent*. This is the aspect of the stimulus being coded such as frequency or intensity. The second is the *transformation*, which corresponds to how referent is represented in the neural output. This encompasses the transduction of synaptic input via specific biophysical cascades that give rise to neural events, a description of how the signal is represented in the characteristics of the events and the fidelity or reliability of this representation. In the present work, a neural event is defined as an isolated action potential, or a burst of action potentials. The third aspect is the *transmission* of the encoded information, pertaining to the anatomical and temporal substrates for information transfer such as axonal pathways and neural networks. Finally, there is an *interpretation* of the coded information by the receiving neurons. This stage is often inaccurately referred to as “decoding”. However, there is ample evidence that neurons filter information or are tuned to specific inputs, thus the message delivered to the receiving neuron is not necessarily an exact replica of the initial signal received by the transmitting neuron. Nonetheless, this final stage is fundamental in establishing a candidate code. Any number of correlations can be made between stimulus

characteristics and neural responses, but if the code cannot be interpreted by the target neural circuits, then the putative code is not the true means of information transfer.

Rate Codes

One of the simplest neural codes is the *rate code* which corresponds to the relationship between a measurable characteristic of the stimulus such as intensity of stretch (Adrian, 1926), or optimal orientation of visual stimuli (Henry, 1974) (*referent*) and neuronal firing rate (*transformation*). Rate codes are often described by frequency-current (F/I) curves or tuning curves. While the firing rate can be simply defined as the number of spikes per unit time, there are a number of variations in the selection of the duration of the time window (Dayan, 2001). For instance, if the stimulus is a steady state depolarization and the neural response is relatively constant throughout the trial, then the rate corresponds to the number of spikes divided by the length of the trial. However, in some cases the stimulus intensity slowly rises and falls and the neuron fires faster or slower in response. Under these conditions, dividing the trial into smaller time windows and calculating the firing rate for each window reveals the time course of the changes in intensity in the changing frequency of the neural response.

Rate codes consist of a series (more than two) of spikes that are *transmitted* along the axon to synapses where the code is *interpreted* through synaptic dynamics. These spikes could elicit excitatory post-synaptic potentials (EPSPs) that simply summate until spike threshold is reached, provided the period of the code was shorter than the duration of the EPSP. Alternatively, rate codes could be interpreted in the frequency dependence of synaptic plasticity demonstrated at the neuromuscular junction and central synapses (Zucker, 1989; Sargsyan, 2004).

Spike Timing Codes

Sometimes the stimulus and the neural response rate fluctuate too rapidly for the average or time dependent firing rate to adequately describe time dependent aspects of the stimulus regardless of the duration of the bin chosen. This is true of Gaussian stimuli with high frequency components. However, if the stimulus is repeated multiple times and the raster plots of spike times are summed in bins, the resulting histogram reveals that the timing of spike events reliably corresponds to the timing of stimulus events (Mainen, 1995; Dayan, 2001). Thus, it is more appropriate to correlate the timing of stimulus events (*referent*) with spike timing (*transformation*).

There are a number of ways to define the spike time. One technique is to identify peaks in the first or second derivative of the membrane voltage and set the time of spike threshold as an arbitrary fraction of this value (Azouz, 1999). A second technique is to choose a voltage level that is supra-threshold for spiking and determine the spike time as the time the action potential upstroke crosses this threshold (Figure 1).

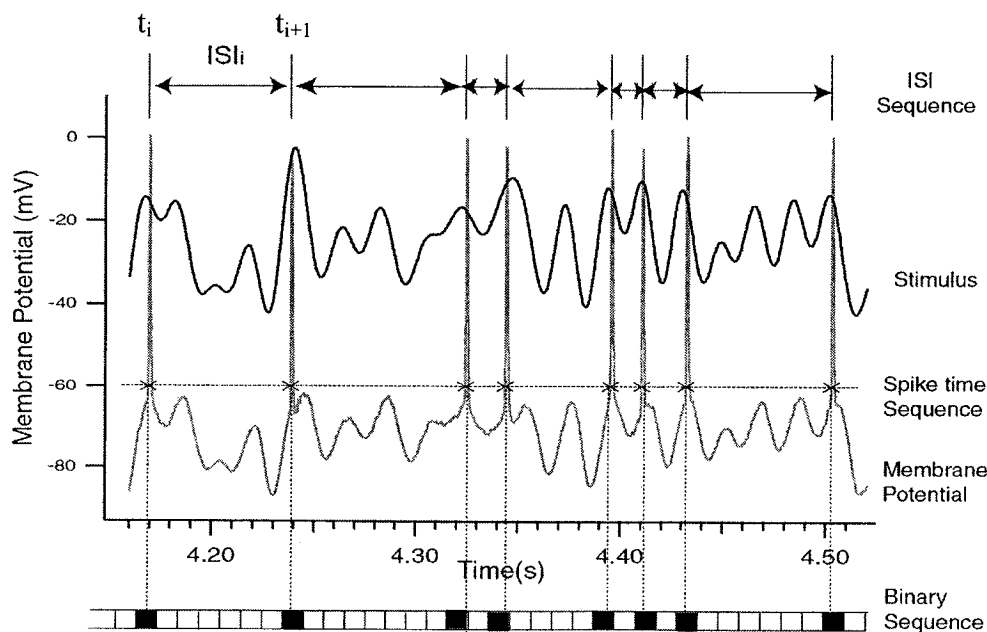


Figure 1: Determining the spike time. The membrane potential (grey) and stimulus (black) are plotted versus time. The timing of action potentials is determined by assigning voltage threshold (horizontal line at -60 mV) and determining the time that the action potential upstroke crosses this threshold. This produces a sequence of spike times ($t_1, t_2, t_3, \dots, t_n$). The inter-spike interval (ISI) sequence is determined by $(t_{i+1} - t_i)$ for each spike time (t_i). The binary sequence is determined by binning time and determining whether a spike occurred in that bin of time. If so, the bin is marked with a 1 and otherwise marked with a 0. In the present example the bins corresponding to spikes are black and the others are white.

Lastly one can bin the membrane potential into windows of time and ask whether or not an action potential occurred in that bin. If an action potential occurred a 1 is assigned to that bin, if not a 0 is assigned. If the bin size is chosen to be sufficiently small (< 2 ms) such that only one action potential occurs per bin this generates a series of zeros and ones. The approximate spike timing is the bin number multiplied by the bin size. This represents the neural output as binary string in time, a common perception of the neural code. The latter two methodologies were sufficient for the analyses presented in this thesis.

Once the spike times are known, the time between spikes can also be obtained. These are the inter-spike intervals (ISI) (Figure 1). The distribution of ISIs between nearest-neighbor spikes are described by the ISI histogram. However, in principle, the ISI between any spike and any other spike in the spike train can also be measured. The inverse of the ISIs ($1/\text{ISI}$) corresponds to the frequencies (Hz) in the spike train.

In the spectral domain, coherence is a measure that correlates frequencies in the spike train with frequencies in the stimulus, (Glaser, 1976; Theunissen, 1995). Thus, coherence is a relative measure of how well the timing of spike train events is matched to the timing of stimulus events. The correlation between the occurrences of specific

electrosensory stimulus events and the timing of pyramidal cell spike train events is explored in Chapter 1.

The exact timing of neural responses with respect to stimulus events is also extensively studied. For instance, auditory neurons fire spikes that are precisely timed with a particular phase of stimulus (Koppl, 1997). Spike timing reliability measures the reproducibility of the timing of a spike train event with repeated presentations of the same stimulus. Spike timing precision is measured by the variability in the spike times from trial to trial (Mainen, 1995; Berry II, 1998). The reliability and precision of neural responses is a quantification of the fidelity with which information is coded in the neural response. These measures are applied and interpreted with respect to ELL pyramidal cell processing in Chapters 2 and 3.

It should also be noted that the importance of spike timing in neural coding extends beyond single cell computation to population responses. In the olfactory system, odors are encoded in the network induced timing of sequential activation of a number of mitral cells (Friedrich, 2001). In the cortex, synchrony in the timing of the responses of two or more neurons may be involved in higher level processing (Stevens, 1998; Theunissen, 2003).

Temporal Encoding

High frequency spike train events such as bursts that do not correspond to stimulus frequencies but correlate with other stimulus characteristics, may code information other than event timing (Theunissen, 1995). This information is temporally *encoded* in the duration of the interval between spikes, termed the inter-spike interval (ISI). A variety of potential ISI codes are described by Perkel and Bullock (1968). One

pertinent example is that the instantaneous frequency of the most recent ISI relays information that signals the amplitude of the response at the crayfish neuromuscular junction (Bittner, 1968). Since the time window for the integration of information is set by the duration of the ISI, this offers a significant advantage in the speed of neural processing as compared to average rate codes that must be integrated over longer time windows. A candidate interval code for the intensity of electrosensory stimuli is described in Chapter 3.

The Electric Sense

The electric sense is an excellent model system for the study of neural coding and sensory processing. Electrosensory stimuli are relatively simple and therefore easily defined and mimicked. Electric fish have demonstrable behaviours in response to both authentic and laboratory mimics of electrosensory stimuli. The neural architecture of low level electrosensory processing regions has been well characterized (Maler, 1974; Maler, 1981, 1991) and is amenable to *in vitro* and *in vivo* studies. Finally, recent cloning and in situ hybridization studies demonstrate a potential for the molecular manipulation (Fernandez, 2003; Harvey-Girard, 2003) of neural responses. The following section is a detailed description of the electrosensory system and processing. The emphasis will be on the electrosensory lateral line lobe (ELL) which is the first processing center of the electric sense and acts as a gateway to higher brain centers.

Electroreception: Bringing the Outside, Inside.

Electric fish are nocturnal and use the electric sense as their primary means of navigation, communication and prey detection. The weakly electric, brown ghost knife fish, *Apteronotus leptorhynchus*, generates a continuous electric field around its body using a

neurally derived electric organ (Kirschbaum, 1983) located in the trunk and tail (Figure 2). This field is a continuous, quasi-sinusoidal, pacemaker driven electric organ discharge (EOD).

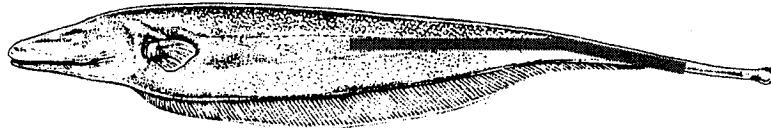


Figure 2: *Apteronotus leptorhynchus*. The male brown ghost knife fish. The electric organ is a neurally derived structure located in the trunk and tail (indicated by grey bar).

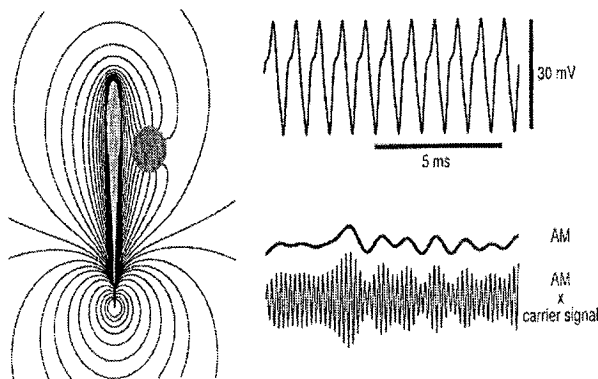


Figure 3: Schematic diagram of the electric organ discharge (EOD). A) The EOD of *A.*

leptorhynchus is a continuous quasi-sinusoidal wave. The EOD frequency ranges by animal between 600 and 1000 Hz. B) Objects entering the EOD field cause distortions or amplitude modulations of the EOD carrier signal. Adapted from Krahe and Gabbiani, 2004.

Electrosensory stimuli distort the EOD carrier wave and are described by changes in EOD amplitude, frequency and phase (Figure 3). There are three identified electrosensory stimuli that result in amplitude modulations (AMs) of the EOD and elicit behavioural responses: communication calls or “chirps”, beat patterns and distortions due to objects that have different impedance properties than the surrounding water.

In *A. leptorhynchus*, the EOD frequency range is approximately 600-800 Hz in females and over 1000 Hz in males (Dunlap, 1998, 2003). The fish can modulate pacemaker output and alter EOD frequency in two behaviourally relevant ways, communication and the jamming avoidance response (JAR) (Heiligenberg, 1991; Zupanc, 2002). Communication calls or “electrical chirps” are transient (<100 ms) increases in EOD frequency that result in 40 to 100’s of Hz AMs. When presented laboratory mimics of a conspecific’s EOD, the fish will chirp in response (Engler, 2001). Alternatively, when two fish are in close proximity to each other, their EODs mix resulting in beat pattern stimuli; depending on the sex of the interacting fish, the beat frequency can range from a few Hertz to >300 Hz. The fish can discriminate the beat frequency, (the difference in frequency between the two EOD frequencies), as well as determine which fish has the higher or lower frequency (Heiligenberg, 1981, 1985). Beat frequencies less than 10 Hz elicit the JAR (Heiligenberg, 1980). This modulation of EOD frequency increases the frequency difference between two fish and reduces the interference of beats with sensory input from objects or prey (Matsubara, 1978). Beat patterns can also elicit chirps and the type of chirp depends on the beat frequency (Bastian, 2001b).

Both chirps and beats are global stimuli in that they affect a large extent of the body surface and can be detected from some distance (Rasnow, 1996). However, since the electric field decays rapidly with distance from the fish’s body, object stimuli must be within a few centimeters of the fish in order to be detected (Rasnow, 1996). Thus, small objects such as crustacean prey produce localized stimuli that affect only a small part of the body. During active electrolocation, the fish will scan an object by swimming back

and forth alongside of it (Lannoo, 1992). This results in low frequency, <10 Hz, localized EOD distortions (Nelson, 1999). Fish use electrolocation to discriminate distance, conducting from non-conducting objects (von der Emde, 1998, 2000) and detect prey up to 3 cm away (MacIver, 2001).

Distortions of the EOD whether due to communication calls, beat interactions or prey change the pattern of the transepidermal voltage causing an electric image on the skin (Rasnow, 1996; Lewis, 2001). This change in voltage is transduced by tuberous electroreceptors into discrete spike trains. There are two types of tuberous receptor, time coders (T-units) and amplitude coders (P-units) (Bastian, 1981a). T-unit receptors are sharply tuned to the EOD and fire one spike per EOD cycle. The spike trains of T-units carry information pertaining to the EOD frequency and phase. Amplitude coders are less precisely phase locked to the EOD (Bastian, 1981a; Kreiman, 2000), and fire probabilistically and are hence, termed P-units. The probability of P-unit firing increases and decreases according to time varying changes in the amplitude of EOD distortions. The distribution of P-units over the body surface is not uniform. On one side of the body, approximately 3000-3500 receptors are located in the head region while the remainder of the body surface is serviced by only 3500-5000 receptors (Carr, 1982). Each receptor innervates a single electrosensory afferent, the cell body of which is located in the ganglion of the anterior lateral line nerve. The information about the frequency and amplitude of AMs is carried in the spike trains of P-unit electrosensory afferents that project to ELL. Another class of receptors, the ampullary receptors, detects low frequency exogenous electric signals. The output from T-units and ampullary receptors will not be considered in this Thesis.

The amount of stimulus information transferred in P-unit spike trains is dependent on both the amplitude of the stimulus (reflected in the firing rate of the neuron) and the frequency of the input. The proportion of the stimulus that can be linearly estimated from a single P-unit spike train can be as high as 0.67-0.82 (the coding fraction- Wessel, 1996; Metzner, 1998). Since the timing of P-unit responses is more variable than T-units, it has been suggested that the remainder of the information can be recovered by averaging the stochastic responses of many P-units (Kreiman, 2000). These studies demonstrate that the electroreceptor afferents encode the majority of the stimulus information in their spike trains.

The Electrosensory Lateral Line Lobe: Gateway to the Higher Brain

Electroreceptor afferents relay sensory input to the electrosensory lateral line lobe (ELL). This hindbrain structure is putatively derived from the rhombic lip, the same embryonic region that gives rise to the other octavolateral structures (medullary auditory and lateral line structures) and the cerebellum. The ELL is divided into medial (MS), centromedial (CMS), centrolateral (CLS) and lateral (LS) segments (Figure 4) (Carr, 1982).

Each segment is a somatotopic map of the ipsilateral body surface. In addition, the ELL is a laminar structure consisting of eight layers. The location of the ELL makes it easily accessible for *in vivo* studies while the segmental/laminar structure is amenable to both *in vitro* slice preparations and *in vivo* studies. A schematic of the segmental and laminar organization of the ELL as well as the circuitry is presented in Figure 5 and will be reviewed in the following sections.

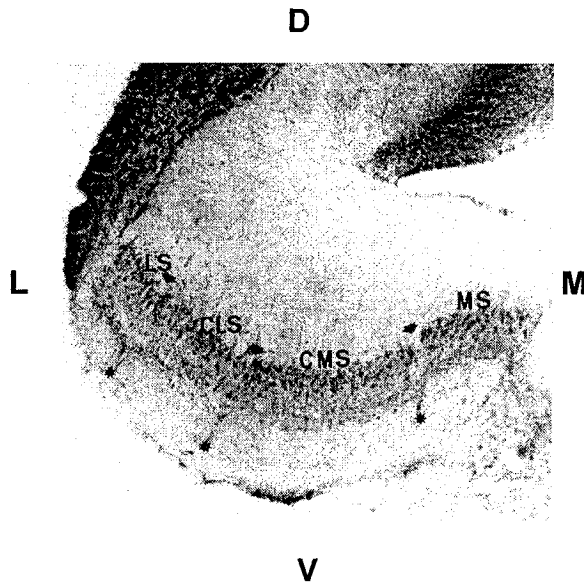
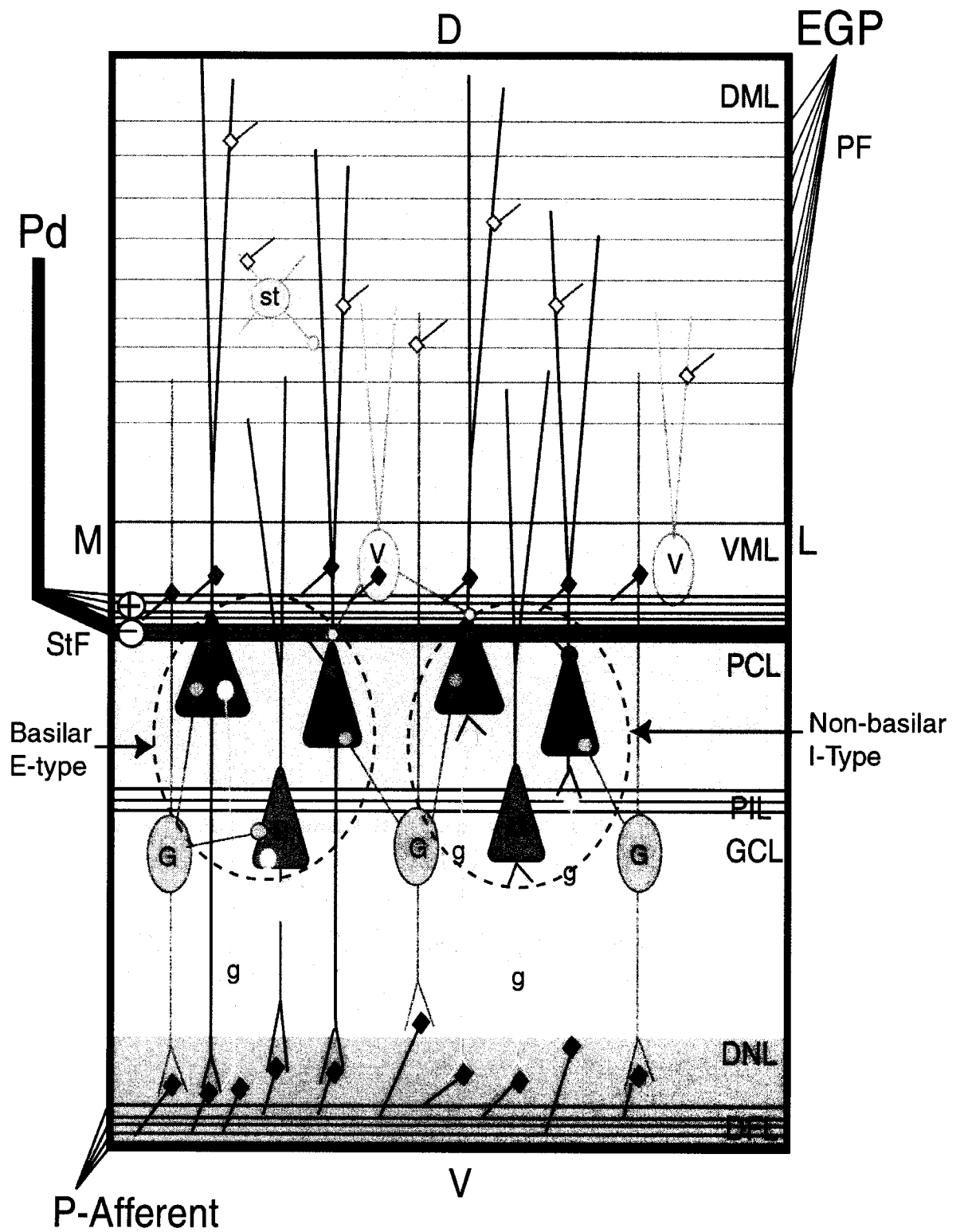


Figure 4: Electrosensory lateral line lobe segments. A coronal section of the ELL shows the segments: medial (MS), centromedial (CMS), centrolateral (CLS) and lateral (LS). P-afferents trifurcate and relay information in triplicate to the CMS, CLS and LS each of which comprise a somatotopic map of the ipsilateral body surface. The MS receives input from the ampullary receptors (not-discussed). Recordings are from pyramidal cells located primarily in CMS, but also CLS and LS.

Figure 5: The laminar structure and cell types of the ELL. Layers: DFL: deep fiber layer, DNL: deep neuropil layer, GCL: granule cell layer, PIL: plexiform layer, PCL: pyramidal cell layer, StF: stratum fibrosum, VML: ventral molecular layer and DML: dorsal molecular layer. Cell types: Pyramidal cells: S-superficial, I- intermediate, D-deep, E-type and I-type are indicated by dashed circles; Interneurons: granule cells (g-type 1, G-type 2); VML cells (V), stellate cells (st). Other abbreviations: Pd: nucleus praeminentialis dorsalis (direct feedback pathway), EGp: eminentia granularis pars posterior (indirect feedback pathway), PF: parallel fibers, M,L,D,V indicate medial, lateral, dorsal and ventral aspects. Excitatory synapses are indicated by diamonds. Inhibitory synapses are indicated by circles. Inputs to the ELL: P-Afferents run medial to lateral (Shumway, 1989) and are excitatory (glutamate); StF (direct feedback) runs medial to lateral and contains both excitatory fibers (+, glutamate) and inhibitory fibers (-, GABA); PF (indirect feedback) runs lateral to medial, excitatory (glutamate).



The tuberous electroreceptor afferents enter the ELL in the deep fiber layer (DFL) and project to the CMS, CLS and LS. In the deep neuropil layer (DNL), P-unit input directly excites basilar pyramidal cells (E-cells) or indirectly inhibits non-basilar pyramidal cells (I-cells) via the excitation of an intermediary granule cell (g). Other granular interneurons (G) in the granule cell layer (GCL) also receive P-unit input and in turn, inhibit both E- and I-type pyramidal cells. The excitatory connections are mediated primarily by glutamatergic synapses involving both γ -amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors (Berman, 1999). P-units can also make gap junction contacts with interneurons (Mathieson, 1987). The inhibitory interneuron connections are mediated by γ -amino-butyric acid (GABA_A) receptors (Berman, 1998b).

Within the pyramidal cell layer (PCL), pyramidal cells are organized in alternating columns of E and I cells (Maler, 1979). Each column is comprised of three cell types: deep, intermediate and superficial. These cell types differ in their dendritic structure and spontaneous and evoked firing properties (Bastian, 2001), projections to higher brain centers (Bastian, 2004) and internal calcium stores (Berman, 1995). Pyramidal cells do not make lateral connections with each other (Maler, 1979). Their axons form the plexiform layer (PIL) and project to the midbrain (torus semicircularis, TS) and the nucleus praeminentialis dorsalis (Pd) via the lateral lemniscus (Carr, 1986). The TS is responsible for higher order projections to brain regions involved in communication (nucleus electrosensorious) and motor control (optic tectum) (Carr, 1981). In contrast the Pd is involved in feedback regulation of the ELL (Bastian, 1996a, 1996b, 1998a).

Pyramidal cells receive feedback from the Pd on their apical dendrites (Berman, 1999). The Pd projects directly to the ELL via the stratum fibrosum (StF) to the ventral molecular layer (VML). The StF consists of axons from glutamatergic, Pd stellate cells and GABAergic, Pd bipolar cells. These synapse in a localized manner on the trunk of the pyramidal cell apical tree and the soma respectively. The Pd also projects indirectly to the ELL via the eminentia granularis pars posterior (EGp) granule cells that extend parallel fibers which traverse the ELL dorsal molecular layer (DML). Parallel fibers are glutamatergic and synapse diffusely over the distal apical tree. Both pathways disynaptically inhibit pyramidal cells by excitatory connections with ELL interneurons, primarily VML and stellate cells but also the apical dendrites of certain granule cells and polymorphic cells.

The synapses between pyramidal cells and the direct and indirect feedback pathways are plastic. The plasticity has both presynaptic and postsynaptic loci. Tetanic stimulation of both pathways yields presynaptic potentiation and post synaptic depression, however the balance between these processes differs in the two pathways. Stimulation of the direct pathway results in potentiation both *in vivo* (Bastian, 1999) and *in vitro* (Wang, 1997, 1998; Oswald, 2002) while the indirect pathway is predominantly depressed (Bastian, 1999; Lewis, 2000). The balance between pre and postsynaptic plasticity can be altered by the anti-hebbian pairing of presynaptic tetani with post synaptic depolarizing and hyperpolarizing inputs, (Wang, 1997; Bastian, 1999) stochastic inputs and the modulation of second messenger systems (Lewis, 2000).

Both feedback pathways play a role in the cancellation of redundant signals such as those due to the fish's own movements (Bastian, 1996a, 1996b). The direct inhibitory

feedback pathway has also been implicated in oscillatory output of the pyramidal cell network in response to communication stimuli (Doiron, 2003, 2004). It has been hypothesized that since the connections between the ELL and Pd are reciprocal, the direct feedback pathway may selectively enhance the responses to weak electrosensory inputs such as prey (Berman, 1999).

Processing Electrosensory Input

Unlike electroreceptor output, pyramidal cell spike trains recorded *in vivo* yield poor linear estimates of amplitude modulations (coding fraction < 0.20, Gabbiani, 1996; Metzner, 1998). These studies demonstrated that pyramidal cell spike trains consist of both spike clusters (bursts) and isolated spikes. E-type pyramidal cells fire bursts or spikes in response to stimulus upstroke events while I-type cells respond similarly to down strokes. In both cell types, bursts were better indicators of stimulus events than isolated spikes. Moreover, pyramidal cell responses demonstrate frequency tuning depending on the spatial extent of the stimulus (Chacron, 2003). When presented local prey-like stimuli, pyramidal cell spike trains are coherent with low frequencies in the stimulus. In response to global, communication stimuli pyramidal cell output is coherent with high frequencies. The mechanism of shifting from low to high frequency tuning based on the spatial extent of the stimulus appears to involve stimulation of the non-classical receptive field. However, it has yet to be determined whether it is the afferent stimulation of granule cells, feedback pathways or both that mediates the switch. Nonetheless, these two studies demonstrate that the ELL selectively pre-processes electrosensory input prior to relaying information to higher brain centers via pyramidal cell output. Thus, the ELL acts as an analogous structure to the lateral geniculate nucleus

of the thalamus which controls the flow of visual information from the retina to the cortex (Sherman, 2001, Krahe, 2004).

Pyramidal cell spike trains are interpreted by the neurons in the torus semicircularis and the Pd. The torus consists of 12 layers and approximately 48 cell types (Carr, 1981). Most of the information relayed from the tuberous electroreceptors via ELL pyramidal cell afferents is routed to layers 4 through 7 (Maler, 1982). Within these layers a number of cell types have been identified. Both spiny and aspiny toral neurons are responsive to EOD amplitude modulations and presence or absence of spines is correlated with the frequency filtering properties of these neurons (Rose, 1993). Furthermore, the synapses between ELL afferents and toral cells are plastic (Fortune, 2001). The potential role of this plasticity in the interpretation of ELL input is discussed in Chapter 1. In addition to projecting to higher brain centers, the neurons of torus project to the Pd. This suggests that the torus may influence the direct and indirect feedback loops between the Pd and ELL, and thus, ELL output.

The Action Potential-The unit of the neural code

Since the main focus of this thesis is the recording, modeling and analysis of pyramidal cell action potentials, the next paragraphs will be a brief discussion of biophysical and mathematical models of the action potential. The Nobel Prize winning work of Hodgkin and Huxley (Hodgkin, 1952, 1952, 1952, 1952) is an excellent example of the marriage between experimental and theoretical science. In a series of well designed experiments they identified and characterized the time course and voltage dependence of the fundamental ion currents involved in action potential generation: sodium (Na^+) and potassium (K^+). In the final paper of this four part series, Hodgkin and

Huxley provide a mathematical description of these currents and quantify the contributions of each during the course of the action potential.

In the first three papers of the series, Hodgkin and Huxley biophysically characterize the sodium and potassium currents (I_{Na} , and I_K) that correspond to the upstroke and down stroke of the action potential respectively. They show ion flow across the membrane is voltage dependent. This dependency was quantified by the driving force ($V_m - V_{ion}$) on the ions where V_m is the potential difference across the membrane. The reversal potential, V_{ion} , is calculated from the Nernst equation: $RT/F(\ln[Ion]_o/[Ion]_i)$ where $[Ion]_o$ and $[Ion]_i$ are the differential concentrations of the ion on either side of the membrane. By measuring the current (I) and the driving force during membrane depolarization, the permeability or conductance (g_{ion}) of an ion was estimated using Ohm's Law, $V=IR$. In this case, the resistance, R , equals $1/g$ consequently $g=I/V$ giving $g_{ion}=I_{ion}/(V_m - V_{ion})$. From these calculations they made the following observations about the Na^+ and K^+ currents. In response to membrane depolarization, the conductance of Na^+ , g_{Na} , initially rises rapidly in a regenerative fashion to a maximum. Prolonged depolarization gradually inactivates the mechanism by which Na^+ ions cross the membrane and g_{Na} declines approximately exponentially. Alternatively, in response to the same depolarizations, g_K rises more slowly and is maintained for long periods of time. From these studies they determined that Na^+ currents can be described by both activation and inactivation kinetics while it is sufficient to describe K^+ currents with activation kinetics alone.

In the final paper of the set, they combine these results in a formal mathematical description of the action potential. The total membrane current can be described by the

capacitive current associated with the charging of the membrane, the ionic currents: I_{Na} , I_K , and finally a passive leak current, I_L , which mainly consists of chloride ions and small amounts of sodium and potassium. The capacitive current is described by $C_M(dV/dt)$ where C_M is the membrane capacity per unit area and dV/dt is the change in membrane voltage with time. The ionic currents are described as $g_{ion}(V_m - V_{ion})$. Both the sodium and potassium currents have time and voltage dependent activation, and the sodium current also inactivates. The corresponding conductance versus time curves for activation are sigmoid shaped. Thus, the mathematical description of I_K includes an activation variable, n , the sigmoid shape of which is described by a first order equation raised to a 4th power. The complicated dynamics of I_{Na} were simplified by using two variables, each of which obeys a first order equation. Like n , the activation variable, m , is raised to the 3rd power while the inactivation variable, h , is a simple exponential decay (power=1). Hodgkin and Huxley referred to the substrates for m , h and n as particles, though it is now known that m , n and h correspond to the proportion of in/active channels at given time and voltage while the power values refer the molecular mechanisms that govern channel gating. Using the gating equation, the activation parameter for potassium was described in terms of the voltage dependent probability of gate opening.

$$\frac{dn}{dt} = \alpha_n(V_m)(1-n) - \beta_n(V_m)n \quad (1)$$

The probability that a gate opens is given by the voltage dependent opening rate $\alpha_n(V_m)$ multiplied by probability of finding the gate closed $(1-n)$. Conversely the probability that a gate closes is given by the probability of finding a gate open, n , multiplied by the closing rate, $\beta_n(V_m)$. An alternate form of Equation 1 is

$$\frac{dn}{dt} = \frac{n_{\infty}(V_m) - n}{\tau_n} \quad (2)$$

where

$$\tau_n(V_m) = \frac{1}{\alpha_n(V_m) + \beta_n(V_m)} \quad (3)$$

and

$$n_{\infty}(V_m) = \frac{\alpha_n(V_m)}{\alpha_n(V_m) + \beta_n(V_m)} \quad (4)$$

Similar equations describe m and h . The gating variables α , β , and τ were estimated by Hodgkin and Huxley from experimental results obtained by calculating the sodium or potassium conductance from current at different levels of depolarization, while m_{∞} and n_{∞} were estimated from the steady state sodium and potassium conductances respectively. All these values were plotted against V to identify functions that relate α and β to membrane potential.

The steady state activation and inactivation curves $n_{\infty}(V_m)$, $m_{\infty}(V_m)$, $h_{\infty}(V_m)$ are sigmoid functions. Thus, the following simplification may be made when α and β are not estimated.

$$n_{\infty}(V_m) = \frac{1}{1 + e^{-(V_m - V_{1/2,n})/k}} \quad (5)$$

Equation 5 follows the general form for a sigmoid function where $V_{1/2,n}$ is the voltage at which activation is half maximum, and k is the slope. In this case, $V_{1/2,n}$, k and τ_n are phenomenologically fit to experimental results. This simplification is made in the ELL pyramidal models discussed in this thesis.

The general form of the Hodgkin-Huxley (H-H) equation for the propagation of an action potential is

$$\frac{a}{2R_2\theta^2} \frac{d^2V}{dx^2} = C_M \frac{dV}{dt} + \bar{g}_K n^4 (V - V_K) + \bar{g}_{Na} m^3 h (V - V_{Na}) + \bar{g}_L (V - V_L) \quad (6).$$

Parameters on the left hand side of the equation account for the velocity of action potential propagation or conduction (θ) along the axon of radius (a) and the specific resistance of the axoplasm (R). This equation was integrated numerically by Hodgkin and Huxley, and the end result was an excellent description of the experimentally measured neuronal action potential, refractoriness, and subthreshold responses.

Neuronal Models of Action Potential Generation

Conductance-Based Models

The Hodgkin-Huxley formalism that describes sodium and potassium conductances during action potential generation is also used to describe the electrical properties of neurons that arise from a variety of ionic conductances (Koch, 1999; Dayan, 2001). These conductance-based models range from single compartment descriptions (simply a soma) to large multi-compartment models that describe extensive dendritic and/or axonal architecture and can accurately reproduce the complex dynamics of real neurons. The membrane voltage for a single compartment model is described as follows:

$$C_M \frac{dV}{dt} = I_{app} + \bar{g}_K n^4 (V_K - V_m) + \bar{g}_{Na} m^3 h (V_{Na} - V_m) + \bar{g}_L (V_L - V_m) + g_{ion} a^k b^l (V_{ion} - V_m) \dots \quad (7)$$

The I_{app} is the external current or stimulus injected into the model and $g_{ion} a^k b^l (V_{ion} - V_m)$ describes any additional ionic current described by activation and/or inactivation parameters as outlined above. The affect of space on membrane potential and action potential propagation is not incorporated in these models. On the other hand, multi-

compartment models that capture the extent of neural processes incorporate cable theory to estimate the membrane potential or action potential propagation with distance similar to Hodgkin and Huxley, 1952.

Integrate and Fire Models

The action potential itself is a relatively stereotyped event with limited variability in height or duration. For this reason, it is speculated that little to no information is transferred in the properties of the action potential; rather it is the timing of action potentials that is important (Perkel, 1968). Much of the complexity in neural models arises from the specific conductances involved in the time course of the action potential. This level of complexity is unnecessary if only spike timing is of specific interest, provided the sub-threshold dynamics that lead to spike threshold are accurately modeled. Integrate and fire models which are at the other end of the spectrum from conductance-based models, do not model action potentials (Dayan, 2001). In these models all active membrane conductances are ignored, only sub-threshold membrane dynamics are modeled. In the case of the leaky-integrate and fire (LIF) model the sole membrane conductance is a leak term described by $g_L(V_m - V_L)$. The model works as a simple resistance-capacitance (RC) circuit and is described by the following equation:

$$c_m \frac{dV}{dt} = -g_L(V_m - V_L) + \frac{I_o}{A} \quad (8)$$

The input current to the model is given by I_o and A is the area of the membrane. If this equation is multiplied by the specific resistance of the membrane, $r_m = 1/g_L$, then $c_m r_m = \tau_m$ where τ_m is the time constant of the neuron. The total membrane resistance is given by $R_m = r_m/A$. The resulting equation is

$$\tau_m \frac{dV}{dt} = V_L - V_m + R_m I_o. \quad (9)$$

There are a number of key characteristics of this model. First V_L is the resting potential of the model and when $I_o = 0$ the model will relax to V_L exponentially with time constant τ_m . Second, when V_m reaches a threshold value, V_{th} , the model “fires an action potential” and V_m is reset to V_{reset} . In fact the model does not fire action potentials at all, but the reset is discontinuous and tractable, providing a “spike” time.

ELL pyramidal cells have been modeled with both conductance-based models and integrate and fire models. The results of each type of model are analyzed in Chapters 1 and 3B respectively.

Neural Burst Firing

The patterning of single-neuron spike trains is governed by intrinsic neural conductances and/or the input the neuron receives. In the former case, a number of stereo-typical patterns have been characterized (Llinas, 1988; Connors, 1990). The latter case will be explored thoroughly throughout the course of this thesis.

It has long been known that repeated presentations of the exact same stimulus do not necessarily yield the exact same neural response each time. This trial to trial variability leads to probabilistic descriptions of neural spike responses. The Poisson model is the simplest model of neural statistics which describes the probability of firing one spike per unit time. An essential feature of this model is that the firing of a spike is independent of the occurrence of other spikes during the response. In addition, the inter-spike interval is also independent on other intervals. For a spike generator obeying a Poisson distribution, a histogram of ISI values obtained from the neural response approximates an exponential distribution (Figure 6A).

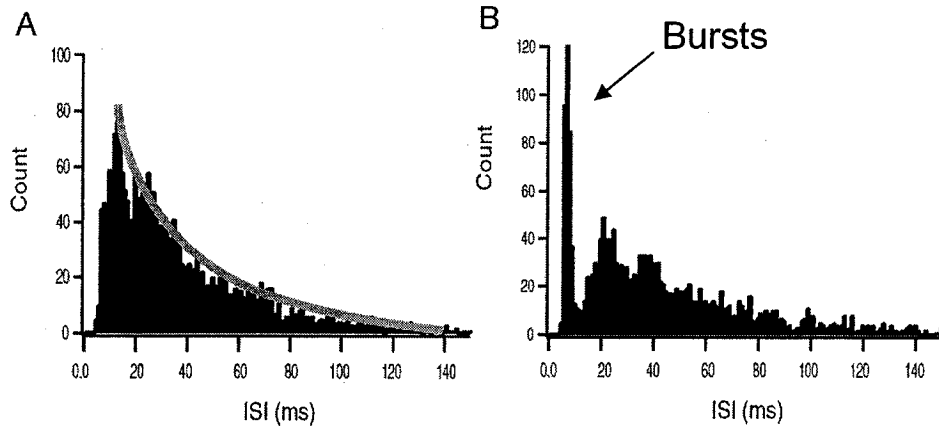


Figure 6: Poisson (A) and Bimodal (B) inter-spike interval distributions. A) The single exponential decay is illustrated by the thick grey line. Histograms are from non-bursting (A) and bursting (B) ELL pyramidal cell spike trains

The spontaneous activity of some electrosensory pyramidal cells can be described by Poisson statistics (Bastian and Nguyenkim, 2001). However, in many cases, during spontaneous and stimulus-driven neural responses (Gabbiani, 1996; Metzner, 1998; Bastian, 2001; Oswald, 2004) clusters of spikes, denoted bursts, with short ISIs occur more often than would be expected from Poisson statistics. The resulting ISI histograms are biphasic or bimodal having sharp peaks at these short ISI values. The decay from these peaks is, at minimum, biphasic and cannot be approximated by a single exponential function (Figure 6B).

The term “burst” is difficult to define. The words bursting and oscillatory activity are sometimes used interchangeably and in a context dependent manner. In the present work, bursts are defined as clusters of spikes that are, at least in part, the result of intrinsic single cell dynamics and occur more often than would be predicted from Poisson statistics. The departure from Poisson statistics is often used to identify burst activity and isolate burst events (Debusk, 1997; Reich, 2000). However, it should be noted that ELL

pyramidal cell ISI histograms can also have non-Poisson peaks at shorter ISIs that are termed oscillatory responses (Doiron, 2003). These responses do not depend on intrinsic single cell dynamics, rather the interaction between the electrosensory network and the stimulus. The Ghostbursts described in this thesis likely do not occur under the same conditions that result in these oscillatory responses (Chapter 3).

The intrinsic biophysical burst mechanisms as well as the patterning of spikes within the burst vary according to the ions involved and/or the locations of the conductances (dendritic or somatic). In a number of systems bursting is a rhythmic alteration of active spiking and quiescent phases. Generally burst generation is dependent on three timescales (Wang, 1995): 1) a relatively fast autocatalytic spike generating process; 2) a slower repolarization process; 3) and an even slower process that sets the duration of quiescence between bursts. For instance, in thalamic relay cells, the burst is initiated when a transient voltage activated calcium current (I_T) depolarizes the neuron (timescale 1) resulting in a barrage of sodium spikes that maintain the level of depolarization long enough for I_T to inactivate (timescale 2). The loss of calcium reduces depolarization and the cell eventually stops firing. During the quiescent state, prolonged hyperpolarization alleviates the inactivation of I_T (timescale 3) and the cycle can begin again (Llinas, 1982; Jahnsen, 1984, 1984b).

Electrosensory pyramidal cell burst responses were first described in Turner et al (1994) and the biophysical mechanism was characterized in Lemon and Turner, (2000). The ELL pyramidal cell burst mechanism is different from than that of the thalamic relay cell and most other identified mechanisms. In response to constant depolarizing current injection, the burst structure (Figure 7) is a series of spikes of decreasing ISI that ride a

ramped membrane potential with a terminating high frequency doublet followed by a large burst (b) AHP. The inter-burst interval is set by the duration of the bAHP.

The biophysical burst mechanism can be reduced to two essential requirements. The first is the *active* backpropagation of somatic spikes via dendritic voltage dependent “spiking” sodium channels. Electrosensory pyramidal cells have sodium and potassium channels along their apical dendrite (Turner, 1994; Rashid, 2001) which support the active backpropagation of somatic spikes along the dendrite. The dendritic spikes differ from the somatic spikes in that they are shorter (mV), broader (ms), lack a significant after-hyperpolarization and have a longer refractory period suggesting different repolarization kinetics than the soma. However, the delayed time course of the dendritic spike with respect to the somatic spike allows the dendritic current to be reflected as a depolarizing after potential (DAP) at the soma. If these channels are blocked by local application of TTX to the proximal dendritic region, backpropagation is prevented and bursting is abolished (Turner, 1994; Oswald, 2004). The exact role of the backpropagation in burst generation varies by mechanism. The inter-play between somatic and dendritic currents is best described as a “ping-pong” effect (Wang, 1999). Somatic spikes backpropagate along the dendrite, “ping”, the extra current from the dendrite sources back to the soma as a DAP, “pong”, and instigates another somatic spike and the cycle repeats (Figure 7A, B). This is the autocatalytic spike generating process.

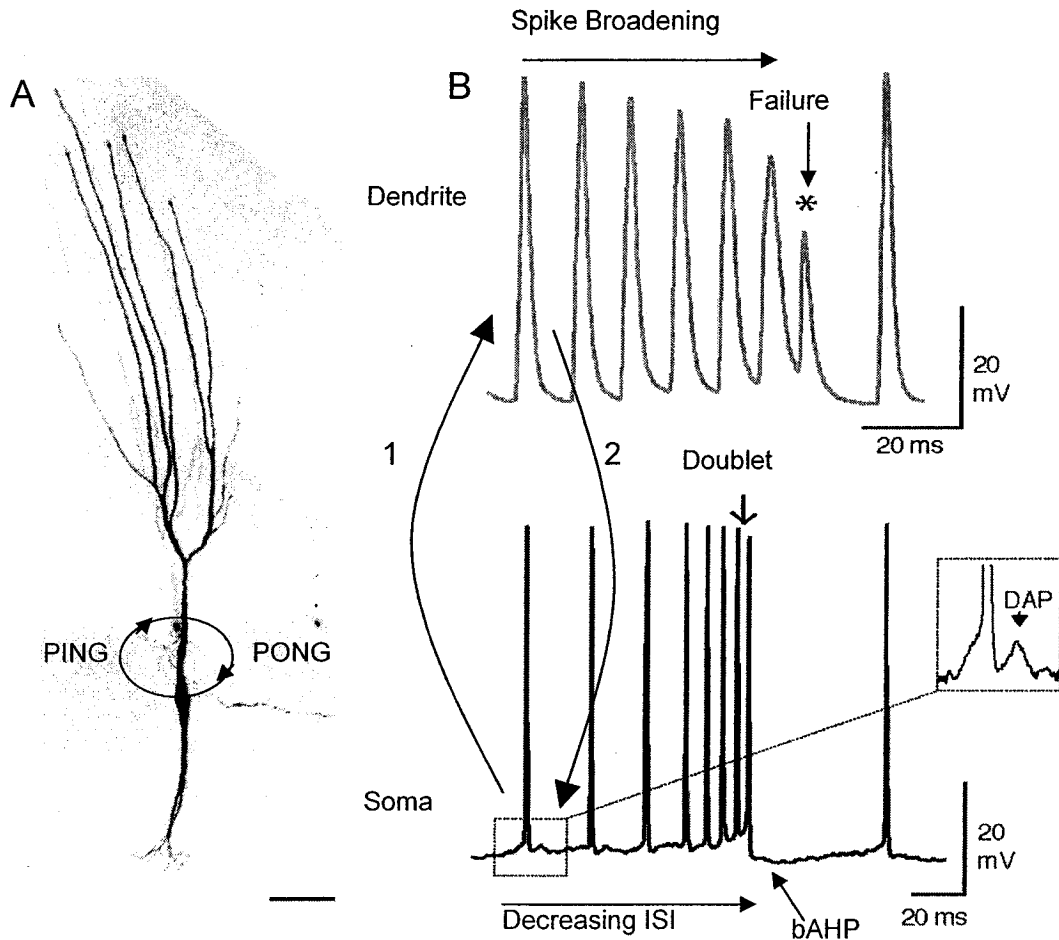


Figure 7: ELL pyramidal cell biophysical burst mechanism. A) ELL pyramidal cell showing a schematic of the PING-PONG interplay between the soma and dendrite. B) Somatic (bottom) and dendritic (top) recordings from two different ELL pyramidal cells illustrate burst mechanism in response to step current injection. The somatic spike backpropagates along the dendrite (1) the broader dendritic spike is reflected at the soma as a depolarizing after potential (DAP, inset). The extra current from the DAP causes the soma to fire additional action potentials with decreasing ISIs. Each subsequent backpropagating spike is broader than the last, potentiating the DAP at the soma. This continues until the DAP elicits a high frequency doublet. The second spike of doublet falls within the dendritic refractory period and the dendrite fails (*) and the burst terminates. Failure at the dendrite allows for a large burst after hyperpolarization (bAHP).

In a number of burst mechanisms the backpropagating spike activates dendritic calcium (thalamic relay cells and hippocampal cells-Magee, 1999) or persistent sodium conductances (Franceschetti, 1995; Brumberg, 2000; Rybak, 2004). These additional

currents are the main contributors to the DAP (or after depolarization, ADP) and autocatalytic spike generation. In hippocampal neurons, the application of TTX abolished spiking but a calcium-dependent ADP could still be induced by direct current injection at the dendrite (Magee, 1999). This suggests that the role of active backpropagation is to transfer a signal from the soma to the dendritic calcium channels that produce the ADP. Bursting in the ELL does not require calcium. Applications of cadmium or nickel do not prevent bursting as in other burst mechanisms (Lemon, 2000). The majority of the current that sources back to the soma as a DAP is sodium ions that enter the dendrite during active backpropagation. Thus, *active* backpropagation and the features of dendritic spike play a more central role in autocatalytic spike generation and burst properties than in other burst mechanisms. Furthermore, since each ensuing spike of the burst is influenced by the DAP produced by the preceding spike, the spikes are not independent as assumed for a Poisson or renewal process. Thus, in this case, identifying bursts by the non-Poisson ISI distribution is justified.

The second requirement for ELL bursting is that active backpropagation is *conditional*, a somatic spike will backpropagate, so long as it does not fall within the refractory period of the dendrite. However, during prolonged depolarization, repetitive somatic spiking causes the dendritic spike associated with backpropagation to broaden (Lemon, 2000) and the dendritic refractory period to become longer (Noonan, 2003). This results in the potentiation of the DAP at the soma. In the early stages of the burst, the extra DAP current enables the soma to reach threshold faster and reduces the ISI. Eventually though, DAP current potentiates to a level such that this current alone is sufficient to generate a somatic spike. The result is a very high frequency doublet with

an ISI so short that the timing second spike of pair falls within the refractory period of the dendrite. The burst terminates because the somatic spike is unable to backpropagate. The resultant loss of the DAP current enables the soma to repolarize with a large burst after-hyperpolarization (AHP).

A number of burst mechanisms involve soma-dendritic interactions, specifically the activation of dendritic conductances by backpropagating somatic spikes. However, a study of thalamic relay cell bursting revealed that slow conductances such as calcium need not be segregated in the dendrites to produce bursts (Destexhe, 1998). Nonetheless, dendritic architecture may play a role in burst production/structure (Mainen, 1998) and regulation (Stuart, 1994; Magee, 1999; Vetter, 2001) in a number of systems. This is true of ELL pyramidal cells, as dendritic size is correlated with spontaneous burst rate (Bastian, 2001). However, what makes evoked ELL bursts unique is that the burst cannot occur without a dendrite. The burst mechanism is dependent on the coupling of two separate, spike generating compartments, soma and dendrite. Since the dendritic dynamics are slightly slower, the dendritic compartment lags the somatic; this interaction both drives and terminates the bursts. The time course of spike broadening throughout a burst provides a potential slow burst variable (Wang, 1995). The mechanism of spike broadening was first investigated using a 153 compartment mathematical model of an electrosensory pyramidal cell (Doiron, 2001). Since calcium channels are not involved in ELL bursting, a number of possibilities were investigated to identify a potential candidate for the slow burst variable. It was shown that a variety of different burst structures could be obtained by incorporating a slow dynamic to either somatic or dendritic conductances. Based on the model results, spike broadening that resulted in a burst structure most like

that seen experimentally was best mediated by a slow cumulative inactivation of a dendritic potassium current.

The large compartmental model described in Doiron et al. 2002 is the most complete description of an ELL pyramidal cell to date. However, this degree of detail is often unnecessary and the model is cumbersome for the description of particular phenomena and analyses. This is true of the dynamical systems analysis of the burst mechanism as well as the response of pyramidal cells to broadband input (Chapter 1,3). For this reason, a simpler, two-compartment model of ELL bursting was developed (Doiron, 2002). This model comprises the two minimal elements for bursting: a dendritic compartment with the “spiking” currents I_{Na} , the delayed rectifier potassium (I_{Dr}) and leak (I_L) that support active backpropagation; and a slow variable, p_d , that represents cumulative inactivation of the dendritic potassium current. This model is used in Chapters 1 and 3B so a short description of the model is presented here.

The model consists of a somatic and a dendritic compartment. The membrane potential in each compartment is described using a modified Hodgkin-Huxley (H-H), conductance based formalism.

Soma:

$$C_m \frac{dV_s}{dt} = I_s + g_{Na,s} \cdot m_{\infty}^2 \cdot (1 - n_s) \cdot (V_{Na} - V_s) + g_{Dr,s} \cdot n_s^2 \cdot (V_K - V_s) + g_{leak} \cdot (V_l - V_s) + \frac{g_c}{\kappa} \cdot (V_d - V_s) \quad (10)$$

Dendrite:

$$C_m \frac{dV_d}{dt} = g_{Na,d} \cdot m_{\infty}^2 \cdot h_d \cdot (V_{Na} - V_d) + g_{Dr,d} \cdot n_d^2 \cdot p_d \cdot (V_K - V_d) + g_{leak} \cdot (V_l - V_d) + \frac{g_c}{1 - \kappa} \cdot (V_s - V_d) \quad (11)$$

The last term in Equations 10 and 11 corresponds to the coupling of the two compartments by electrotonic diffusion of current from the soma to the dendrite and vice versa. The activation and inactivation variables are described by:

$$\frac{dn_s}{dt} = \frac{n_\infty(V_s) - n_s}{\tau_{n,s}} \quad (12)$$

$$\frac{dn_d}{dt} = \frac{n_{\infty,d}(V_d) - n_d}{\tau_{n,d}} \quad (13)$$

$$\frac{dh_d}{dt} = \frac{h_{\infty,d}(V_d) - h_d}{\tau_{h,d}} \quad (14)$$

$$\frac{dp_d}{dt} = \frac{p_{\infty,d}(V_d) - p_d}{\tau_{p,d}} \quad (15)$$

where the subscripts d and s refer to dendrite and soma respectively.

In order to recreate the essential characteristics of *in vitro* bursts in the simplest manner the model differs somewhat from the classic H-H description. Two approximations reduce the description of the somatic membrane potential (V_s) from a four dimensional H-H representation, to a two dimensional system. First, the sodium activation parameter, m_∞ , tracks voltage instantaneously. Second the somatic inactivation parameter, h , is set to $(1-n)$. The latter approximation was justified by observations in the large compartmental model that $h_s + n_s \approx 1$ (Doiron et al, 2001, 2002).

Since experimental studies suggest that pyramidal cell burst dynamics are of dendritic origin, the burst parameters are incorporated in the dendritic description of this model. Like the somatic compartment, the activation of sodium in the dendritic compartment tracks V_d . However, since the dendritic refractory period is longer than that of the soma (Lemon, 2000), the inactivation of dendritic sodium channels is described by h_d which has a longer time constant ($\tau_{h,d}$) than n_d . The slow cumulative inactivation that

leads to spike broadening and DAP potentiation was modeled by incorporating a slow inactivation variable, p_d , in the dynamics of the delayed rectifier potassium channel. The time constant of p_d is much slower (5 ms) than m , n or h (≤ 1 ms) and the model adequately recreates burst responses.

There are two major assumptions of this model. First, the dendritic current responsible for slow cumulative inactivation is unknown. While describing the delayed rectifier with the additional parameter, p_d , is sufficient and justified (see Doiron, 2002), other potassium channels such as members of the $K_{V3,3}$ family may be involved (Rashid, 2001; Fernandez, 2003). Second, the dendrite is modeled as a point neuron. The model does not account for the cable properties of the dendrite and the ensuing changes in the height and width of the dendritic spike with distance from the soma (Williams, 2000; Waters, 2004). The loss of these spatial effects result in an inadequate description of the time course of the DAP and limit the use of this model for studies of bursting in neural coding (Chapter 3B).

The Role of Bursts in Neural Processing

Bursts may play a unique role in neural processing for two reasons. First, both intrinsic and network dependent burst mechanisms have different threshold requirements than isolated spikes and thus, bursts comprise a unique group of spike train events that may correspond specific sensory inputs or coding units. Second, bursts are high frequency, multi-spike events that evoke different synaptic dynamics than isolated spikes.

Studies of bursting in the thalamus and ELL suggest that burst events are indicators of the occurrence of specific stimulus events (for reviews see: Sherman, 2001; Krahe, 2004). Specifically, thalamic bursts in the lateral geniculate nucleus (LGN) have

been associated with the detection of near threshold visual stimuli (Guido, 1995) and the onset salient stimulus features (Martinez-Conde, 2002; Lesica, 2004; Alitto, 2005). In the thalamic medial geniculate nucleus, burst responses are correlated with the optimal frequency of auditory stimuli (Massaux, 2004). At the cortical level, V1 bursts may code stimuli that have strong spatial opponence (Reich, 2000) and the optimality of the stimulus orientation (Debusk, 1997; Martinez-Conde, 2002). Cortical bursts may code additional stimulus information in their structure. For instance, the number of spikes in a burst is correlated with the optimality of the stimulus orientation (Debusk, 1997) and with the slope of the stimulus (Kepecs, 2003). In the ELL, pyramidal cell bursts are better indicators of stimulus up or down strokes than isolated spikes (Gabbiani, 1996; Metzner, 1998), signal the occurrence of low frequency stimulus events (Oswald et al, 2004, Chapter 1) and code additional information about stimulus intensity in the burst inter-spike interval (Chapter 3).

In addition to roles in sensory processing, bursts are involved in other forms of neural processing. In the zebra finch, individual neurons of the high vocal center (HVC) code specific events in the song sequence (Hahnloser, 2002). There is mounting evidence that HVC neurons fire sequentially and transfer song information via bursts to subpopulations of neurons of the premotor, robust nucleus of the archistriatum (RA). The RA neurons then relay this information via stereotyped burst sequences to the motor neurons that control singing. Bursts have also been implicated in initiating wide spread recurrent activity in cortical layer 4 (Beierlein, 2002) and in the synchronization of layer V pyramidal neurons (Silva, 1991). Finally, burst firing is involved in central pattern

generator networks that are responsible for respiratory rhythms in mammals (Rybak, 2003) and a number of behaviours in invertebrates (Skelton, 1992).

Interpreting the Information in Burst Responses

Burst firing may be an advantageous means of information transfer for the simple reason that “two spikes are better than one”. Single action potentials often fail to produce postsynaptic responses at central synapses (Stevens, 1995; Goda, 1997). Sequential activation of presynaptic terminals by high-frequency multi-spike bursts could enhance synaptic transmission through calcium dependent short-term facilitation, increasing the probability of synaptic release at some time during the burst (Lisman, 1997). A complementary hypothesis is that interactions between synaptic facilitation and depression result in optimized EPSP amplitudes for information transfer when burst ISIs of a resonant frequency arrive at the presynaptic terminal (Izhikevich, 2003). The authors extend this hypothesis by demonstrating that burst ISIs may also be resonant with membrane potential oscillations. Thus, bursts may selectively transfer information according to burst ISI depending on the status of the postsynaptic cell. Another study demonstrates that high frequency thalamocortical bursts can bypass the feed forward inhibition of cortical pyramidal cells by interneurons and elicit recurrent network activity (Beierlein, 2002). High frequency multi-spike inputs promote the temporal summation of EPSPs in both excitatory and inhibitory neurons. Since excitatory neurons have longer time constants and more prominent NMDA currents, excitation wins over inhibition both in single cells and the network. This may enhance information transfer by the selective amplification of high frequency inputs. Finally, stimuli that elicit thalamic bursts incorporate a period of hyperpolarization that deinactivates the calcium currents that

underlie burst firing (Llinas, 1982; Lesica, 2004; Alitto, 2005). This leads to a period of quiescence in spontaneous thalamic activity which enables postsynaptic cortical cells to recover from depression and enhances the efficacy of the first burst spike (Swadlow, 2001). Ensuing burst spikes further ensure transmission and may elicit multi-spike responses in the postsynaptic cell.

With regard to electrosensory processing, it is presently unknown how the information in ELL bursts is interpreted by higher brain centers. Speculations about the roles that synaptic plasticity (Fortune, 2001), membrane properties and network interactions may play in the selective transfer of information relayed by bursts will be discussed in Chapters 1, 3 and the Conclusion of this thesis.

Overview of Signal Detection Theory and Pattern Classification

In Chapters 1 and 3 the methodology of the feature extraction measure described by Metzner et al. (1998) is used extensively. This measure is derived from signal detection theory and uses pattern classifiers to determine which sensory input patterns give rise to spike train events (Gabbiani, 1996). Signal detection and pattern classification techniques quantify the degree to which signals can be discriminated based on the neural response (Glaser, 1976). This section will review material from texts and literature that describe the methodology and interpretation of these techniques (Glaser, 1976; Gabbiani, 1996; Metzner, 1998; Dayan, 2001). The figures and examples are drawn from the responses of electrosensory pyramidal cells but are meant solely for illustrative purposes.

Signal Detection

In Figure 1A, a stimulus of time varying current (I , nA, grey trace) is presented to a neuron. The neural response is represented by the spike times with respect to the stimulus as indicated in the upper trace (black) of Figure 1A. A stimulus event is defined as an upstroke followed by a down stroke. The goal is to quantify how well the occurrence of stimulus events can be discriminated based on the spike output of the neuron. It is easy to see that there is a high certainty that high amplitude events ($I > 0.75$ nA, $V_m \gg \text{threshold}$) will produce spikes (Figure 1A). There is also a high certainty that low amplitude events will not yield spikes ($I < 0.3$ nA, V_m is sub-threshold). However, mid-range amplitudes sometimes produce spikes and sometimes do not, even though the input current appears to be supra-threshold (indicated by arrows, Figure 1A). Thus, one

would be uncertain of whether or not a stimulus event occurred based on the neural response. The variability in the neural response arises from the fact that the threshold for spike generation is dependent on the membrane voltage (V_m) which is influenced by both the input and the state of the neuron. In cases of high amplitude current inputs, the influence of the inputs dominate the membrane voltage and a spike is certain (vice versa for very low amplitude inputs, when a non-spike event is certain). Inputs that result in near threshold membrane voltages may be out-competed by the influence of the state of the cell making spike events uncertain. These regions of certainty (C) and uncertainty (U) are defined “by eye” and illustrated by the dashed horizontal lines in Figure 1A. The peak amplitudes of all stimulus events can be divided into two distributions: amplitudes that gave rise to spikes and amplitudes that did not result in spikes. Hyperpolarizing stimulus events ($nA < 0$, down strokes followed by upstrokes) are not included in these distributions. These distributions can be approximated by Gaussian fits as shown in Figure 1B (spikes: black, no-spikes: grey). Each distribution is described by a mean (μ) and a standard deviation (σ) as indicated. The regions of un/certainty are indicated by the hatched line under the x-axis. Note that the region of uncertainty approximately corresponds to the region of overlap between the two distributions.

Since the neural response for a given event is one of two possibilities: a spike or no spike, a highly discriminating neuron would be expected to reliably spike only in response to stimulus events that were supra-threshold for spiking and otherwise remain quiescent. This would increase the distance between the two distributions (spike and no-spike) and maximize regions of certainty (non-overlapping) and minimize the regions of uncertainty (overlapping).

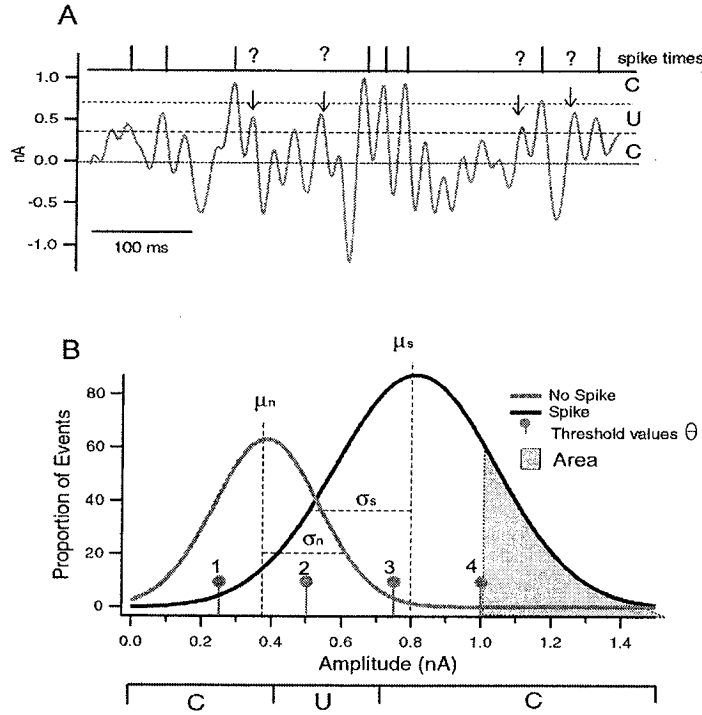


Figure 1: Discrimination of events based on spike train responses. A) Spike times (upper trace) in response to a stimulus with a time varying current (nA) intensity (grey). One stimulus event is defined as an upstroke followed by a down stroke. Arrows and question marks indicate stimulus events that did not give rise to spikes, even though events of similar amplitude produced spikes at other times in the trial. The dashed lines indicate regions of certainty (C) or uncertainty (U) in neural responses. B) Distributions of peak amplitudes of stimulus events that either gave rise to spikes (black) or did not produce spikes (grey). Events with amplitudes less than 0 nA are considered hyperpolarizing and never elicit spikes and are not included. Each distribution is described by a mean (μ) and a standard deviation (σ) as indicated. The amplitudes corresponding to regions of certainty and uncertainty in the neural response (A) are indicated below the x-axis. Not that the region of uncertainty corresponds to the regions of overlap for the two distributions. Grey circles and the shaded grey area correspond to threshold values (θ) described in the text and Figure 2.

A simple measure of the distance between the two distributions is:

$$d' = \frac{(\mu_S - \mu_N)^2}{1/2(\sigma_S^2 + \sigma_N^2)} \quad (1)$$

High d' values correspond to separable distributions with minimal overlap; that is, the differences in the means are greater than the sum of the variances. Thus, d' is a simple measure of discrimination.

In the present example, the ability to discriminate whether or not a stimulus event occurred based on the neural response depends on the amplitude of the event. This dependency is quantified in terms of four possible outcomes; 1) the *probability of correct detection* (P_D) which is the probability that there was an event and the neuron spiked; 2) the *probability of false alarm* (P_{FA}) which is the probability that the neuron spiked in the absence of an event; 3) the *probability of false negatives* which is the probability that there was an event and the neuron did not spike ($1-P_D$); and the probability that there was no event and no spike was elicited ($1-P_{FA}$). The P_D and P_{FA} for each amplitude can be obtained by sliding a moving threshold along the x axis of Figure 1B (examples of threshold values are indicated by grey circles) and integrating the area under each distribution to the right of this threshold (see threshold 4, shaded region corresponds to the area of integration, Figure 1). The P_D for a given threshold value corresponds to the area under the “spikes” distribution and the P_{FA} is the area under the “no-spikes” distribution. These are plotted against each other to produce the Receiver Operating Characteristic curve (ROC) (Figure 2).

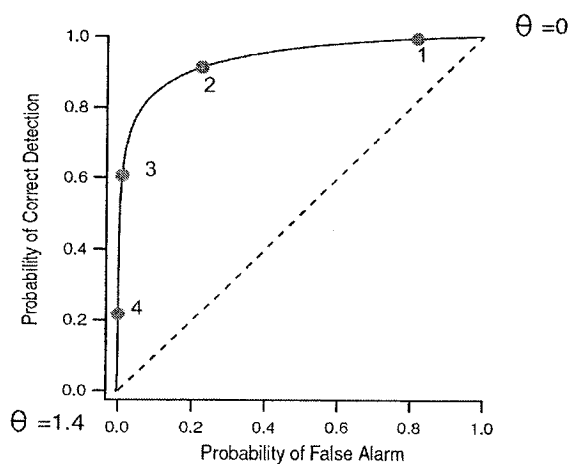


Figure 2: The Receiver Operating Characteristic (ROC) curve. The probability of correct detection is plotted against the probability of false alarm. The dashed line corresponds to chance detection. The curve is parameterized by the amplitude threshold (θ). The P_D and P_{FA} values that correspond to the amplitude threshold values are indicated by grey circles in Figure 1 are shown (grey circles marked 1-4). The curve traces from high (1.4 nA) to low (0 nA) threshold values from left to right.

The dashed line corresponds to chance where there is an equal probability of correct detection or false alarm. The curve is parameterized by the threshold (θ) chosen. At very low thresholds (θ near zero) there is a very high probability of correct detection but also a very high probability of false alarm. This gives rise to points in the upper right hand corner (point 1, Figure 2). When the threshold is very high corresponding to very high amplitude stimuli, P_{FA} is zero and the curve is only dependent on the area under the spikes distribution (point 4-Figure 1 and 2).

In Figure 1, the threshold value for point 2 falls within the uncertain region when the distributions overlap. In Figure 2 it can be seen that there is a very high probability that the neuron will fire in response to an event of greater magnitude than threshold and low probability of false alarm for values near threshold. This suggests that the discriminability of the neuron for events greater than spike threshold is fairly high. This can be quantified in a single overall error rate (ϵ) by taking the minimum of the curve defined by plotting the P_{FA} versus the probability of error (P_ϵ):

$$P_\epsilon = \frac{1}{2}P_{FA} + \frac{1}{2}(1 - P_D) \quad (2)$$

where $(1-P_D)$ is the probability of false negatives (Figure 3). The maximum value of ϵ is 0.5 which corresponds to chance detection. This is determined by the choice of $\frac{1}{2}$ as the coefficient for P_{FA} and $(1-P_D)$. This coefficient corresponds to the most uncertain one can be about whether or not a spike will occur; a guess would be correct 50% of the time. This upper bound on ϵ can be illustrated by considering the two extreme threshold conditions. First at a threshold equal to zero, the $P_{FA}=1$ and $P_D=1$ (see Figure 2) and $P_\epsilon=$

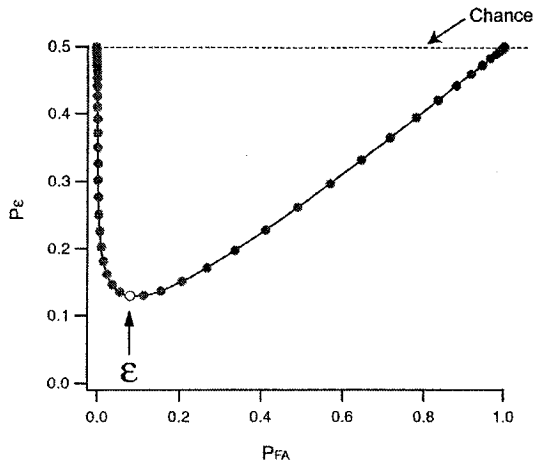


Figure 3: The probability of error

(P_ϵ). The error rate (ϵ) is calculated as the minimum P_ϵ calculated from $\frac{1}{2}P_{FA} + \frac{1}{2}(1-P_D)$ at each threshold value. P_ϵ is plotted against P_{FA} to show the minimum (ϵ) marked by the open white circle. The dashed line across the top of the graph corresponds to chance (0.5) which is the maximum value ϵ can take.

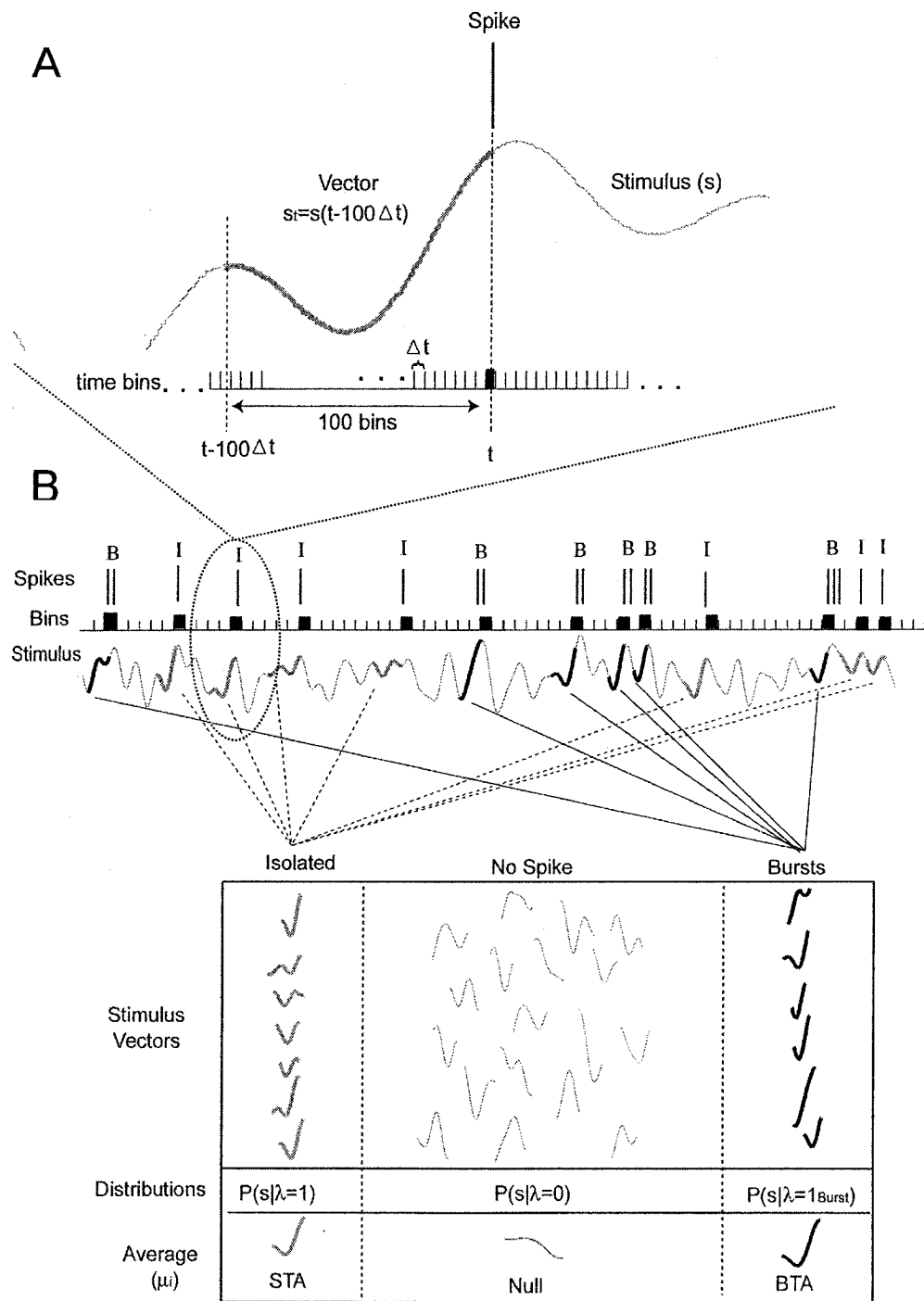
$\frac{1}{2}(1) + \frac{1}{2}(1-1) = 0.5$. Second, at very high thresholds (∞), $P_{FA}=0$ and $P_D=0$ (furthest most point on the tail of the distribution) thus, $P_\epsilon = \frac{1}{2}(0) + \frac{1}{2}(1-0) = 0.5$. The error rate corresponds to the minimum error achieved in predicting the occurrence of a spike by an ideal observer who has no knowledge of the probability of spiking (Poor, 1994). Low ϵ values correspond to high discriminability. This can be interpreted as a measure of how well a spiking neuron can convey information about the presence or absence of signals in the stimulus.

Pattern Classification and Feature Extraction

Pattern classification schemes use the same principles as signal detection schemes. The difference is that detection schemes ask, “Given a response, is a signal present?” And classification schemes ask, “What are the characteristics of the signal that gave rise to a known response?” In the electrosensory system this has been used to identify upstrokes or down strokes as stimulus features that are discriminated in the stimulus waveform by E or I-type pyramidal cells respectively (Gabbiani, 1996; Metzner, 1998). This section is an interpretation of the methods described in Metzner et al, 1998 and used extensively in Chapters 1 and 3.

Typically there are three types of electrosensory pyramidal cell responses of interest: isolated spikes, bursts and no response (null). The questions are, “What stimuli give rise to these responses?” and “Based on the responses, how well are they discriminated?” The stimuli were identified in the following manner. The stimulus, $s(t)$, and the spike train were binned in time. The bin size (Δt) was determined such that each bin only contained one spike train event (burst or isolated spike). If a bin (t) contained a spike train event, the corresponding stimulus waveform that preceded that bin was the 101 dimensional (D) vector $s_t = (s(t-100 \Delta t))$ (Figure 4A) Based on the spike train event elicited, the stimulus waveforms (s) were divided into the distributions: isolated spikes- $P(s|\lambda=1_{\text{isol}})$; bursts- $P(s|\lambda=1_{\text{bursts}})$; and $P(s|\lambda=0)$ which corresponds to the null (101D vectors preceding bins with no spikes) (Figure 4B). The means of these distributions were 101D vectors that corresponded to the average stimulus that elicited a spike (spike triggered average, STA), a burst (burst triggered average, BTA) or no event (Figure 4B). For brevity, this overview will focus only the stimuli that gave rise to isolated spikes- $P(s|\lambda=1)$ versus null- $P(s|\lambda=0)$; the same analysis was conducted for burst events.

Figure 4: Determining the stimulus vectors and the vector distributions. A) The stimulus region (light grey, thin) corresponding to the circled region in B is enlarged to show the details of a single stimulus vector (dark grey) that elicited a spike at time t . The stimulus, s , was binned in time, bin size ($\Delta t=3-15$ ms) and the corresponding stimulus waveform that preceded that bin t was the 101 dimensional vector $s_t = (s(t-100 \Delta t))$. B) Based on the spike train event elicited, burst (B), isolated spike (I) or no spike, the stimulus waveforms (s) were divided into the distributions: bursts- $P(s|\lambda=1_{\text{bursts}})$; isolated spikes- $P(s|\lambda=1_{\text{isol}})$; and $P(s|\lambda=0)$ which corresponds to the null. The means of these distributions are a vector that corresponds to the average stimulus that elicited a spike (spike triggered average, STA), a burst (burst triggered average, BTA).



In order to assess discriminability of isolated spikes, the distributions $P(s|\lambda=1_{\text{isol}})$ versus $P(s|\lambda=0)$ must be first maximally separated in stimulus space. Since the stimulus waveforms are 101 dimensional vectors they are not described by a single parameter such as amplitude as in the previous example. Plotting the distributions (as in Figure 1) in a 101 dimensional space is inconceivable. Likewise, measuring the distance between the distributions using equation would also be extremely difficult. However, the description of each stimulus vector can be reduced to a single value by the projection of the stimulus vector, s_t , onto an optimal feature vector, f , (also 101 dimensions) (Box 1).

Box 1: Vector Projection The vectors can also be thought of as 1x N matrices defined by 1 row and N columns. The transpose (T) of a 1x N matrix is a N x 1 matrix: N rows, 1 column. The dot product of the 1 x 3 matrices, A and the transpose of B, denoted B^T, yields a single value.	A: 4 5 6 B: 7 8 9 $A \bullet B = A \cdot B^T = \sum_{a=1}^N A_a B_a$ $= 4 \cdot 7 + 5 \cdot 8 + 6 \cdot 9$ $= 122$
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The projection distributions are defined by projecting each stimulus vector, s_t , onto the optimal feature, f , and comparing to a threshold θ :

$$h_{f\theta}(s) = f^T \cdot s - \theta \quad (3)$$

When $f^T \cdot s$ is greater than threshold $h_{f\theta}(s) > 0$, s is assigned to the projection distribution $P(f^T \cdot s | \lambda = 1)$: stimuli that elicit a spike, otherwise s is assigned to $P(f^T \cdot s | \lambda = 0)$: stimuli that do not elicit spikes.

The optimal feature that best separates the projection distributions is determined by using Fisher's linear discriminant function to maximize the signal to noise ratio (SNR):

$$\text{SNR}(f) = \frac{[f^T \bullet (m_1 - m_0)]^2}{f^T \bullet \left(\frac{1}{2} \Sigma_1 + \frac{1}{2} \Sigma_0 \right) \bullet f} \quad (4)$$

The means of the distributions $P(s|\lambda=1)$ and $P(s|\lambda=0)$ are m_1 , and m_0 respectively and Σ_1 and Σ_0 are the covariances of these distributions as defined by:

$$\Sigma_i = \langle (s - m_i) \bullet (s - m_i)^T \rangle_i \quad (5)$$

The brackets, $\langle \dots \rangle_i$, denote the average over the distribution $i=0, 1$.

A significant proportion of the mathematical methodology for feature extraction is devoted to determining the optimal feature. The methods of Lagrange multipliers and the diagonalization of matrices used in Metzner et al., 1998 are generally described in linear algebra and calculus textbooks (for examples: Nicholson, 1995) so the discussion of these techniques will be limited.

The vector f that maximizes the SNR was obtained by the method of Lagrange multipliers. This method provides a solution for f by maximizing the numerator of Equation 4 and keeping the denominator constant. In this the case the solution for f is

$$\frac{1}{2}(\Sigma_1 + \Sigma_0) \bullet f = m_1 - m_0 \quad (6)$$

$$f = 2(\Sigma_1 + \Sigma_0)^{-1} (m_1 - m_0). \quad (7)$$

One misconception is that the optimal feature, f , is the simply the STA. In Equations 4-7 the m_1 is the STA. Note that in Equation 7, the feature is determined by subtracting the average of the null distribution (m_0) from the STA and then multiplying

by $2(\Sigma_1 + \Sigma_0)^{-1}$. Thus, the feature is the difference of the means of the distributions scaled by the variance around these means. In circumstances when a zero mean stimulus is used (as in Chapters 1, 3), m_0 is near zero for most points. The optimal feature can look very similar to the STA, but they are not equivalent entities (Figure 5).

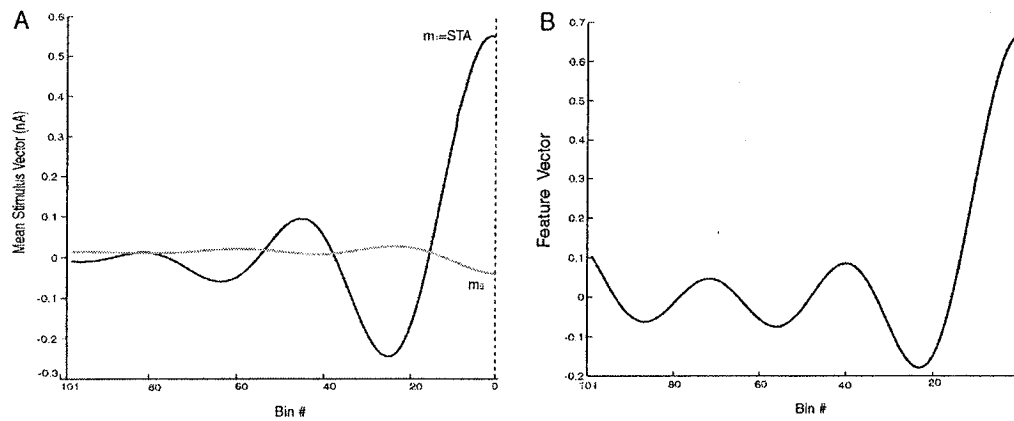


Figure 5: The spike triggered average and the optimal feature. A) The spike triggered average (black) and null average (grey) plotted against bin number. The time of spike or non-event is at bin 0. B) The optimal feature that best separates the spike and null distributions.

In this case, the mean stimulus waveform that elicits a spike is a large upstroke preceded by a small hyperpolarization while that which does not elicit spikes is relatively flat (Figure 5A). The feature vector that separates the distributions classifies the upstroke as the key feature (pattern) for predicting whether or not a spike will occur (Figure 5B).

If the covariance matrices, Σ_1 and Σ_0 are invertible: $\Sigma_i \Sigma_i^{-1} = I$, where I is the identity matrix (ones on the diagonal and zeros elsewhere); Equation 7 can be solved immediately. However, typically the matrices are not easily invertible and Equation 7 is solved in a common subspace where they are invertible. This is accomplished by diagonalizing the matrix $\frac{1}{2}(\Sigma_1 + \Sigma_0)$ numerically. This returns a series of eigenvalues, λ_i ,

(definition of λ differs from that used in the probability distributions) and corresponding eigenvectors, e_i that characterize the matrix.

To calculate the optimal feature, the eigenvalues and corresponding eigenvectors were organized in order of decreasing magnitude and the first n eigenvalues that accounted for 99% of the variance were retained. Then $(m_1 - m_0)$ was projected onto each of the corresponding eigenvectors, e_i .

$$v_i = (m_1 - m_0)^T \cdot e_i \quad (8)$$

The optimal feature, f , was obtained from

$$f = \sum_{i=1}^n \frac{v_i}{\lambda_i} e_i. \quad (9)$$

Once the feature (pattern classifier) has been identified all that remains is to determine how well upstrokes can be discriminated by isolated spikes. This is accomplished using the signal detection techniques outlined in the previous section. As stated previously, the projection of s_i onto f^T reduces the description of the stimulus waveform to a single parameter which is compared to a threshold (Eqn. 3) to determine the projection distributions: $P(f^T \cdot s | \lambda=1)$ and isolated spike is predicted, and $P(f^T \cdot s | \lambda=0)$ a null event is predicted. These distributions can be plotted in a 2 dimensional plane (Figure 6).

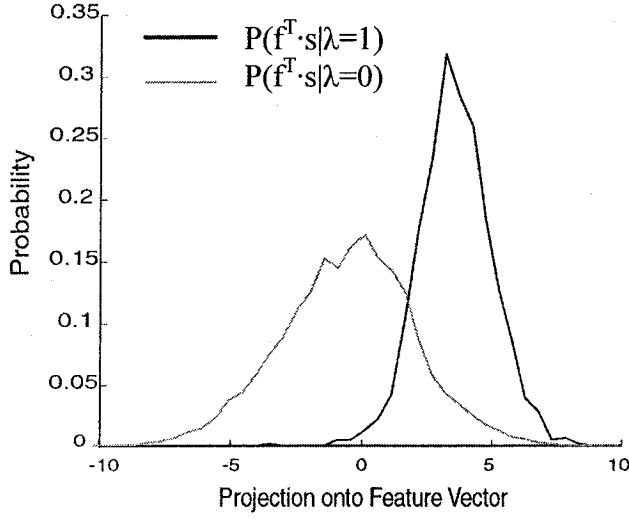


Figure 6: The projection distributions.

The distributions obtained by projecting s_i onto f^T . The probability of predicting a spike based on s_i is given by $P(f^T \cdot s | \lambda = 1)$, black line, and the probability of predicting a null event (no spike) is given by $P(f^T \cdot s | \lambda = 0)$, grey line.

Determining a feature vector that maximizes the SNR is akin to maximizing the distance between the means of the projection distributions, $P(f^T \cdot s | \lambda = i)$, $i=0,1$ and minimizing the sum of the variances (d' , Eqn. 1). The means and variances of the projection distributions are denoted by μ_i , and σ_i^2 respectively. Each element of the projection distributions is defined by the projection, $f^T \cdot s$, and μ_i is the average of these projections.

$$\mu_i = \langle f^T \cdot s \rangle_i = f^T \cdot m_i \quad (10)$$

This is equivalent to projecting the feature onto the mean (m_i) of the conditional distribution $P(s | \lambda = i)$ (most right hand side of eqn.10). Referring to the calculation of d' (Eqn. 1), the numerator is $(\mu_1 - \mu_0)^2$, thus,

$$(\mu_1 - \mu_0)^2 = [f^T \cdot m_1 - f^T \cdot m_0]^2 \quad (11)$$

$$= [f^T \cdot (m_1 - m_0)]^2 \quad (12)$$

Equation 12 is the numerator of Equation 4. Similarly, the variances of these distributions are given by,

$$\begin{aligned}
\sigma_i^2 &= \left\langle \left[\mathbf{f}^T \bullet (\mathbf{s} - \mathbf{m}_i) \right] \left[(\mathbf{s} - \mathbf{m}_i) \bullet \mathbf{f}^T \right] \right\rangle_i \\
&= \mathbf{f}^T \bullet \Sigma_i \bullet \mathbf{f} .
\end{aligned} \tag{13}$$

Substituting these into the denominator of Equation 1 gives the denominator of Equation 4.

$$\begin{aligned}
\frac{1}{2}(\sigma_1^2 + \sigma_0^2) &= \frac{1}{2}(\mathbf{f}^T \bullet \Sigma_1 \bullet \mathbf{f} + \mathbf{f}^T \bullet \Sigma_0 \bullet \mathbf{f}) \\
&= \mathbf{f}^T \bullet \left(\frac{1}{2} \Sigma_1 + \Sigma_0 \right) \bullet \mathbf{f}
\end{aligned} \tag{14}$$

Following this, the feature extraction performance can be quantified by the ROC curve described in the previous section. The probability of correctly identifying a stimulus vector as eliciting a spike, P_D , and the probability of false alarm, P_{FA} for successive threshold values is calculated by taking the corresponding areas under the projection distributions, $P(\mathbf{f}^T \cdot \mathbf{s} | \lambda = i)$ (Figure 7). In addition, the probability of misclassification, P_e , and the error rate, ϵ , can also be calculated as previously described.

In Chapter 3, I expand on the feature extraction technique using a series of optimal features to separate burst distributions of different inter-spike interval (ISI) values. The error rate for discriminating between ISI distributions is the basis of the interval coding measure described.

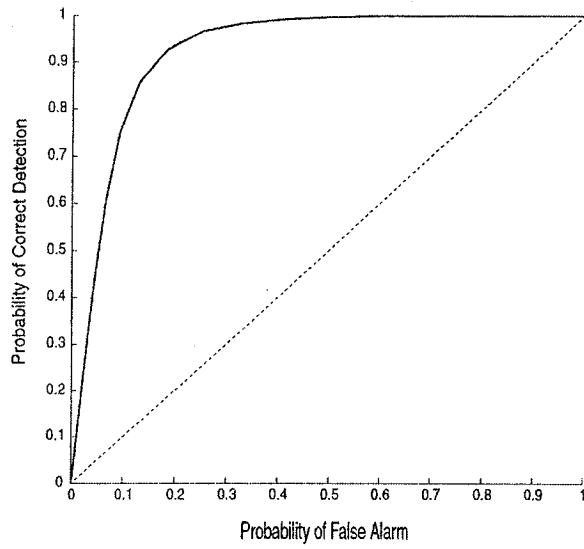


Figure 7: Quantification of the performance of the linear classifier $\mathbf{h}_{f,\theta}(\mathbf{s})$. The ROC curve: the probability of correctly identifying a stimulus waveform as eliciting a spike versus the probability of false alarm for successive thresholds ($\mathbf{h}_{f,\theta}(\mathbf{s}) = \mathbf{f}^T \cdot \mathbf{s} - \theta$). Generally, isolated spikes are good detectors of stimulus upstrokes.

Chapter 1

Parallel Processing of Sensory Input by Bursts and Isolated Spikes

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Division of Labor:

A.M. Oswald:	<i>In vitro</i> data collection and analysis Interpretation of results, all text and figures
M.J. Chacron:	Development of analysis code Analysis of <i>in vivo</i> data Interpretation of results
B. Doiron:	Ghostbuster Model Simulations Interpretation of results
J. Bastian:	<i>In vivo</i> data collection

Abstract

Burst firing is commonly observed in many sensory systems and is proposed to transmit information reliably. Although a number of biophysical burst mechanisms have been identified, the relationship between burst dynamics and information transfer is uncertain. Electrosensory pyramidal cells have a well-defined, back-propagation dependent burst mechanism. We employ *in vivo*, *in vitro* and modeling approaches to investigate pyramidal cell responses to mimics of behaviorally relevant sensory input. We find that within a given spike train bursts are biased toward low frequency events while isolated spikes simultaneously code for the entire frequency range. We further demonstrate that burst dynamics are essential for optimal feature detection but are not required for stimulus estimation. We conclude that burst and spike dynamics can segregate a single spike train into two parallel and complementary streams of information transfer.

Introduction

During sensory processing, peripheral input is processed so that relevant aspects of the sensory signal are encoded in the sequences of action potentials that constitute the neural code. In theory, if the stimulus input and the neural output are known, then the transformation between the two defines the coding mechanism. However, due to the complexity of most sensory input and the variability of neural responses, only a few of the mechanisms by which stimuli are represented in neural codes have been identified (Rieke, 1997; Eggermont, 1998; Lestienne, 2001)

Action potential sequences can consist of single spikes, high frequency spike clusters known as bursts, or both. When coding for comparable signals, bursts, in comparison to single spikes, have been shown to improve the signal to noise ratio (Sherman, 2001), and transmit specific or additional information about the stimulus (Reinagel, 1999; Keat, 2001; Kepecs, 2002; Martinez-Conde, 2002). Bursts have been further suggested to enhance the reliability of information transfer between neurons (Malinow, 1994; Lisman, 1997). However, since the behavioral significance of specific stimulus patterns is often unknown; it is difficult to determine if bursts code for particular categories of relevant stimuli that are distinct from those coded for by single spikes (Krahe, 2004).

Behaviorally relevant electrosensory inputs have been characterized and easily mimicked (Zupanc, 1993; MacIver, 2001). Specifically, prey stimuli are low frequency (<10 Hz) inputs while communication stimuli are often high frequency (>40 Hz). The ability of electrosensory pyramidal cells to encode these stimuli depends on both the frequency content and the spatial extent of the stimulus (Bastian, 2002; Chacron, 2003). *In vivo* studies of electrosensory pyramidal cell bursting have revealed that spontaneous burst production varies with dendritic structure and spontaneous firing rate (Bastian, 2001), and stimulus evoked bursts extract stimulus features better than single spikes (Gabbiani, 1996; Metzner, 1998; Bastian, 2001). In addition, pyramidal cells can switch from a non-oscillatory to oscillatory and burst firing pattern contingent on spatial stimulus characteristics (Doiron, 2003). However, the mechanism underlying burst production was not addressed in these studies.

In vitro studies have established that the biophysical burst mechanism of electrosensory pyramidal cells is dependent on the active dendritic backpropagation of somatic spikes (Turner, 1994; Lemon, 2000) and theoretical analyses have further characterized these burst dynamics (Doiron, 2001, 2002; Laing, 2003; Laing, 2003). These previous studies suggest that the electrosensory system is an ideal preparation in which to elucidate the role of a biophysically and dynamically characterized burst mechanism in sensory coding.

This study addresses bursting and stimulus encoding in the weakly electric fish, *Apteronotus leptorhynchus*. Using both *in vivo*, and *in vitro* experiments and an accurate burst model (Ghostbuster) we demonstrate that bursts exclusively code for low frequency stimuli, while isolated spikes code for high frequency stimuli. We further demonstrate that optimal burst coding is dependent on burst dynamics. Thus, the electrosensory system creates two “streams” of frequency specific information that could be differentially decoded by higher brain centers.

Methods

In vivo: The preparation and surgical techniques for recording from *Apteronotus leptorhynchus* have been previously described (Bastian, 1996a, b) and were in accordance with the University of Oklahoma animal care and use guidelines. Stimulation consisted of random amplitude modulations of the animal’s own EOD produced by adding an amplitude modulated train of sinusoidal signals (EOD mimic) to the animal’s normal discharge. The RAMs were produced by multiplying the sinusoids with band-limited Gaussian noise (0-60 Hz bandwidth, 8th order Butterworth filter) and applied with local geometry (Bastian, 2002) using a small dipole (2 mm tip spacing) at a distance

2-3 mm lateral to the skin surface. Extracellular and intracellular recordings of 33 pyramidal cells were obtained primarily from the centrolateral (CLS) and lateral (LS) ELL segments. Extracellular single-unit recordings were made with metal filled electrodes and intracellular recordings were made with 40-100 M Ω , KCl filled micropipettes. Recordings were pre-amplified and acquired with Cambridge Electronic Design 1401plus hardware and Spike II software.

In vitro: Transverse brain slices from the ELL of *A. leptorhynchus* were prepared in accordance with animal care protocols of the University of Ottawa as previously described (Berman, 1997). The slices were maintained at room temperature (20-22°C) in oxygenated ACSF for 1-2 hours prior to recording. The ACSF contained (in mM) 124 NaCl, 3 KCl, 0.75 KH₂PO₄, 2 CaCl₂, 2 MgSO₄, 24 NaHCO₃, 10 D-Glucose, all chemicals provided by Sigma.

The pyramidal cell layer is located just below the stratum fibrosum (StF), an easily identifiable landmark in the ELL slice (Berman, 1997). Intracellular recordings from 37 ELL pyramidal cells from the entire pyramidal cell layer were made using 80-120 M Ω borosillate glass electrodes, pulled by a Brown-Flaming P-87 puller (Sutter Instrument, CA) and filled with 2 M KAc. Of these cells, 24 were identified as bursting neurons by direct current injection and analyzed.

Recordings were obtained primarily from centromedial segment (CMS). Differences in oscillatory and burst activity across the ELL segments have been reported both *in vitro* (Turner, 1996) and *in vivo* (Metzner, 1998). Consequently a comparison of 16-CMS and 6-CLS and 2-LS *in vitro* pyramidal cell responses was conducted. The cells showed no significant differences in any parameters (such as firing rate, membrane

potential, and coherence etc.) studied, including bursting and consequently the data were pooled.

Upon impalement, the neurons were allowed to stabilize for ~5-10 minutes. If necessary, a constant 0.1-0.5 nA hyperpolarizing current was injected through the recording electrode to stabilize the cells. A series of depolarizing current steps were injected to determine the current at which the firing rate was approximately 10-20 Hz (0.1-0.5 nA), which is within the range of spontaneous activity *in vivo*.

Previous studies have used Gaussian noise stimuli to stimulate the receptive fields of pyramidal cells *in vivo* (Bastian, 2002; Chacron, 2003, Doiron 2003a). The membrane potential of pyramidal cells can follow these fluctuations (Chacron, 2003) presumably because electroreceptor EPSPs are brief (Berman, 1998b). We therefore injected similar noise signals via the recording electrode into pyramidal cells *in vitro*. The noise stimuli were Gaussian noise with cut off frequencies of 0-60, 0-20 or 40-60 Hz and standard deviation of 0.5 nA, unless otherwise indicated. This was added to a 100 s depolarizing current (0.1-0.5 nA, as determined above). Stimulation was computer-controlled (IgorPro and Pulse Control, Wavemetrics, OR) (Herrington, 1995), and pyramidal cell responses were amplified (Axoclamp 2A, Axon Instruments, Burlingame CA), sampled at 10 KHz and stored for analysis (Igor Pro and Pulse Control).

Drug application: Tetrodotoxin (15 μ M in ACSF, Sigma) was focally pressure ejected in the ventral molecular layer above the Stf in a region corresponding to the apical dendrites of the pyramidal cells (Turner et al, 1994).

The model neuron: The previously described two-compartment model of an ELL pyramidal cell known as the ghostbuster model (Doiron, 2001, 2002) contains all the

essential elements to reproduce bursting as detailed by Lemon and Turner (2000) and Doiron et al, (2001). The model neuron is comprised of an isopotential soma and a single dendritic compartment that are joined through an axial resistance of $1/g_c$ allowing for the electrotonic diffusion of currents from the soma to dendrite and vice versa. Both compartments contain the essential spiking currents; fast inward Na^+ ($I_{\text{Na},s}$, $I_{\text{Na},d}$) and outward delayed rectifying (Dr) K^+ ($I_{\text{Dr},s}$, $I_{\text{Dr},d}$), and passive leak currents (I_{leak}) (in the above and hereafter, the subscripts s and d denote soma and dendrite respectively). The presence of spiking currents in the dendrite enables the active backpropagation of somatic action potentials required for bursting. The membrane potentials at the soma, V_s , and the dendrite, V_d , are determined using a Hodgkin-Huxley-like formalism (Koch, 1999). The original Ghostbuster model (Doiron, 2002) comprised six non-linear differential equations; this study expands on this model to incorporate persistent sodium currents ($I_{\text{NaP},s}$, $I_{\text{NaP},d}$), and a slower activation of spiking sodium in the dendrite. The input current $I_s(t)$ delivered to the soma was comparable to the zero-mean, Gaussian noise (0-60 Hz) that was added to constant depolarizing current I_0 and injected *in vitro*. $I_s(t)$ was produced by a fourth order Butterworth filter and the standard deviation ($\sigma = 3.7 \mu\text{A}/\text{cm}^2$) of $I_s(t)$ and the intensity of the offset current I_0 ($0.21 \mu\text{A}/\text{cm}^2$) were adapted so that the firing rate (32 Hz) and burst fraction (0.24) were similar to the *in vitro* recording seen in Figure 1. V_s and V_d are described by the following equations.

$$C_m \frac{dV_s}{dt} = I_0 + I_s(t) + g_{Na,s} \cdot m_{\infty}^2 \cdot (1 - n_s) \cdot (V_{Na} - V_s) + g_{NaP,s} \cdot m_{\infty,NaP,s}^2 \cdot (V_{Na} - V_s) \\ + g_{Dr,s} \cdot n_s^2 \cdot (V_K - V_s) + \frac{g_c}{\kappa} \cdot (V_d - V_s) + g_{leak} \cdot (V_l - V_s) \quad (1)$$

$$C_m \frac{dV_d}{dt} = g_{Na,d} \cdot m_d^2 \cdot h_d \cdot (V_{Na} - V_d) + g_{NaP,d} \cdot m_{\infty,NaP,d}^2 \cdot (V_{Na} - V_d) \\ + g_{Dr,d} \cdot n_d^2 \cdot p_d \cdot (V_K - V_d) + \frac{g_c}{1 - \kappa} \cdot (V_s - V_d) + g_{leak} \cdot (V_l - V_d) \quad (2)$$

The parameter g is a maximal conductance ($g_{\max}$, mS/cm²), while m and s are activation parameters and h , n and p are inactivation parameters. As previously described (Doiron et al, 2002), the activation of spiking Na⁺ in the soma, $m_{\infty,s}$, instantaneously tracks the membrane voltage, V_s , and its inactivation, h_s , is modeled as $h_s \approx 1 - n_s$ where n_s is the activation parameter for $I_{Dr,s}$ described by:

$$\frac{dn_s}{dt} = \frac{n_{\infty}(V_s) - n_s}{\tau_{n,s}} \quad (3)$$

In order to simplify the Ghostbuster model, the activation of dendritic spiking Na⁺ was originally slaved to the dendritic voltage as described for $I_{Na,s}$. This resulted in burst ISIs that were less than 3 ms, which were rarely seen experimentally. Immunocytochemical and electrophysiological data suggests that spiking Na⁺ channels are confined to active zones along the proximal apical dendrite. This type of channel distribution can lead to delays in DAP production that are not accounted for in the ghostbuster due to the single compartment dendrite. Thus, we describe the activation of spiking Na⁺ in the dendrite, m_d , by:

$$\frac{dm_d}{dt} = \frac{m_{\infty,d}(V_d) - m_d}{\tau_{m,d}} \quad (4)$$

This resulted in doublet ISIs that were 4-6 ms and closer to experimental values (5-8 ms).

The inactivation of spiking Na^+ h_d in the dendrite, as well as the activation n_d and the slow inactivation p_d of the dendritic K^+ currents are modeled as described in Doiron et al. (2002).

$$\frac{dh_d}{dt} = \frac{h_{\infty,d}(V_d) - h_d}{\tau_{h,d}} \quad (5)$$

$$\frac{dn_d}{dt} = \frac{n_{\infty,d}(V_d) - n_d}{\tau_{n,d}} \quad (6)$$

$$\frac{dp_d}{dt} = \frac{p_{\infty,d}(V_d) - p_d}{\tau_{p,d}} \quad (7)$$

Persistent sodium (NaP) currents may modulate bursting (Doiron, 2003) thus, for completeness and a better fit to experimental data, we incorporate NaP currents in both the soma and the dendrite as described in Doiron et al. (2001). This increased the excitability of model neuron and lowered bias current required to quantitatively reproduce *in vitro* results. For simplicity, we slave the activation of NaP, m_{NaP} , to the membrane voltage in both the soma and the dendrite.

The voltage dependence of each activation and inactivation parameter is described by steady state conductance curves as presented for the activation of somatic spiking Na^+ , m_{∞} :

$$m_{\infty}(V) = \frac{1}{1 + e^{-(V - V_{1/2})/k}} \quad (8)$$

The values for $g_{\max}$, τ , and $V_{1/2}$ and k for each current are given in Table 1. Other parameter values are: the ratio of somatic to total area: $\kappa = 0.6$; the reversal potentials: $V_{\text{Na}} = 40$ mV, $V_K = -88.5$ mV, $V_{\text{leak}} = -70$ mV; membrane capacitance: $C_m = 1$ $\mu\text{F}/\text{cm}^2$; $g_{\text{leak}} = 0.22$ mS/cm^2 and $g_c = 0.21$ ms/cm^2 . In some simulations, $g_{\text{Na,d}}$ was set to zero to remove active dendritic spiking and prevent bursting. This does not affect NaP conductance.

Equations 1 through 7 were integrated in time via a fourth order Runge-Kutta approximation scheme with a time step of 25 μs . All simulations were 200 s of simulation time.

Current	$g_{\max}$ (mS/cm^2)	$V_{1/2}$ (mV)	k (mV)	τ (ms)
$I_{\text{Na,s}}(m_{\infty,s}(V_s))$	55	-40	3	N/A
$I_{\text{NaP,s}}(m_{\infty,s}(V_s))$	0.14	-58.5	6	N/A
$I_{\text{Dr,s}}(n_s(V_s))$	20	-40	3	0.39
$I_{\text{Na,d}}(m_d(V_d)/h_d(V_d))$	3.1	-40/-52	5/-5	0.3/4.0
$I_{\text{NaP,d}}(m_{\infty,d}(V_d))$	0.1	-58.5	6	N/A
$I_{\text{Dr,d}}(n_d(V_d)/p_d(V_d))$	35	-40/-65	5/-6	6/15

Table 1: Model Parameter Values. Both somatic (s) and dendritic (d) compartments contain the essential spiking currents; fast inward Na^+ ($I_{\text{Na,s}}$, $I_{\text{Na,d}}$) and outward delayed rectifying (Dr) K^+ ($I_{\text{Dr,s}}$, $I_{\text{Dr,d}}$) as well as persistent Na^+ ($I_{\text{NaP,s}}$, $I_{\text{NaP,d}}$). The parameters for the steady state conductance curves for each current are the maximum conductance ($g_{\max}$), the half de/activation voltage ($V_{1/2}$), de/activation time constant (τ) and the de/activation slope (k). In the case of two entries under $V_{1/2}$, τ , and k , the first value corresponds to the activation parameter and the second value to the deactivation parameter.

Data Analysis: *In vivo*, *in vitro* and model results were analyzed using Igor Pro and MATLAB (Mathworks, Natick, MA). The first second of the spike train was not analyzed to exclude transients resulting from the onset of the stimulus. We determined ISI criteria to identify burst ISIs based on the valleys of a bimodal ISI histogram (10 ms, *in vitro*, model neuron) and a previously used criterion for deviations from Poisson statistics (Bastian and Nguyenkim, 2001) (12 ± 4 ms, *in vivo*). Generally ISIs less than 10 ms are in agreement with backpropagation dependent burst ISIs reported *in vitro* (Noonan, 2003). Using the ISI criteria the spike trains were separated into three different spike trains for analysis. The first, denoted “all spikes” was the raw spike train that contained the timing of all spikes. The second contained the timing of only the first spike of the bursts, denoted “burst events”. The final was denoted “isolated spikes” and contained only the timing of spikes that were not burst spikes. The coherence $C(f)$ between the three individual spike trains and the stimulus was calculated according to $C(f) = |P_{sx}(f)|^2 / [P_{ss}(f)P_{xx}(f)]$, where P_{ss} and P_{xx} are the power spectra of the stimulus and the spike train respectively and P_{sx} is the cross spectrum between the stimulus and the spike train. Coherence takes a value between 0 and 1 and is a measure of the strength of the neural response to a stimulus at a given stimulus frequency (f).

Burst- and Spike-Triggered Averages: We determined 400 ms regions of the stimulus centered on each isolated spike or burst event for the *in vivo* and *in vitro* spike trains to be the spike and burst triggered averages (STA/BTA) respectively. In order to assess the specificity of spikes or bursts, we re-injected 0.5 Hz trains of STAs or BTAs with an offset current of +0.1 nA into a small number of pyramidal cells *in vitro*.

Feature Detection: We used the feature extraction technique described in detail by Metzner et al. (1998). Briefly, the spike train and stimuli were binned at $\Delta t = 0.5$ ms so that no more than one spike occurred per bin. For each bin, the stimulus vector was determined as the stimulus waveform preceding the bin $[t-\Delta t; t]$. These vectors were then grouped as distributions according to whether a spike occurred- $P(s|\lambda = 1_{\text{all}})$, a spike occurred and it was an isolated spike- $P(s|\lambda = 1_{\text{isol}})$, a spike occurred and it was the first spike of a burst- $P(s|\lambda = 1_{\text{burst}})$, or a spike did not occur (null) $P(s|\lambda = 0)$. The means (m_i), which are the spike, isolated spike-, burst- and null-triggered averages respectively, as well as the covariances (Σ_i) of the distributions were estimated. Fisher's linear discriminant function was used to determine optimal feature vector (f) to maximize the signal to noise ratio (SNR).

$$\frac{1}{2}(\Sigma_0 + \Sigma_1) \cdot f = m_1 - m_0 \quad (9)$$

$$SNR = \frac{[f^T \cdot (m_1 - m_0)]^2}{f^T \cdot \left(\frac{1}{2}\Sigma_0 + \frac{1}{2}\Sigma_1 \right) \cdot f} \quad (10)$$

The probability of correct detection, P_D , (correctly classifying a stimulus vector as eliciting or not eliciting a spike) and the probability of false alarm, P_{FA} , (misclassifying a stimulus vector as eliciting or not eliciting a spike) can then be determined. The separations between each of the distributions $P(f^T \cdot s|\lambda = 1_{\text{all}})$, $P(f^T \cdot s|\lambda = 1_{\text{burst}})$ and $P(f^T \cdot s|\lambda = 1_{\text{isol}})$ and null distribution $P(f^T \cdot s|\lambda = 0)$ were assessed using these techniques and the receiver operating characteristic (ROC) curve (P_D versus P_{FA}) was plotted for each case.

Statistics: The statistical significances of the results were assessed using paired *t*-tests.

Results

Pyramidal cell burst responses to noise stimuli applied *in vivo* and *in vitro*

The stimulus consisted of broadband Gaussian noise (0-60 Hz) presented as amplitude modulations (AMs) of electrosensory input *in vivo* and intracellular current injection *in vitro*. The mean firing rates in response to the stimulus were similar *in vitro* (18 ± 4 Hz) and *in vivo* (22 ± 13 Hz) and the spike trains were qualitatively similar in that they consisted of isolated spikes as well as high frequency clusters of 2-4 spikes (arrowheads) (Figure 1 A1, B1). The majority of ISI histograms both *in vivo* and *in vitro* had initial sharp peaks at the shortest intervals (Figure 1 A2, B2) and could not be fit by a single exponential ($\chi^2 > 500$); thus the spike train statistics are non-Poissonian, suggestive of burst discharge.

Based on the ISI histograms (Figure 1 A2,B2), an ISI criterion was established to identify spikes belonging to bursts (Turner, 1994, 1996; Debusk, 1997; Metzner, 1998; Reinagel, 1999). A criterion ISI of 10 ms was chosen for *in vitro* data. Since *in vivo* pyramidal cells show a wide range of firing frequencies, criterion ISI's were individually chosen for each cell (see *Methods*). The mean ISI criterion *in vivo* was 12 ± 4 ms. The burst fraction, defined as the total number of spikes contained within bursts divided by the total number of spikes, was determined for each cell and the means were 0.49 ± 0.16 , *in vivo* and 0.22 ± 0.12 , *in vitro*. The burst criterion was then used to separate the full spike train into two component trains: "bursts" that consisted of the timing of the first spike in a burst event, regardless of the number of spikes in a burst, and "isolated spikes" that consisted of the timing of the remaining single spikes.

The coherence between each of the event trains (all spikes, burst events, and isolated spikes) and the stimulus was calculated (Figure 1 A3, B3).

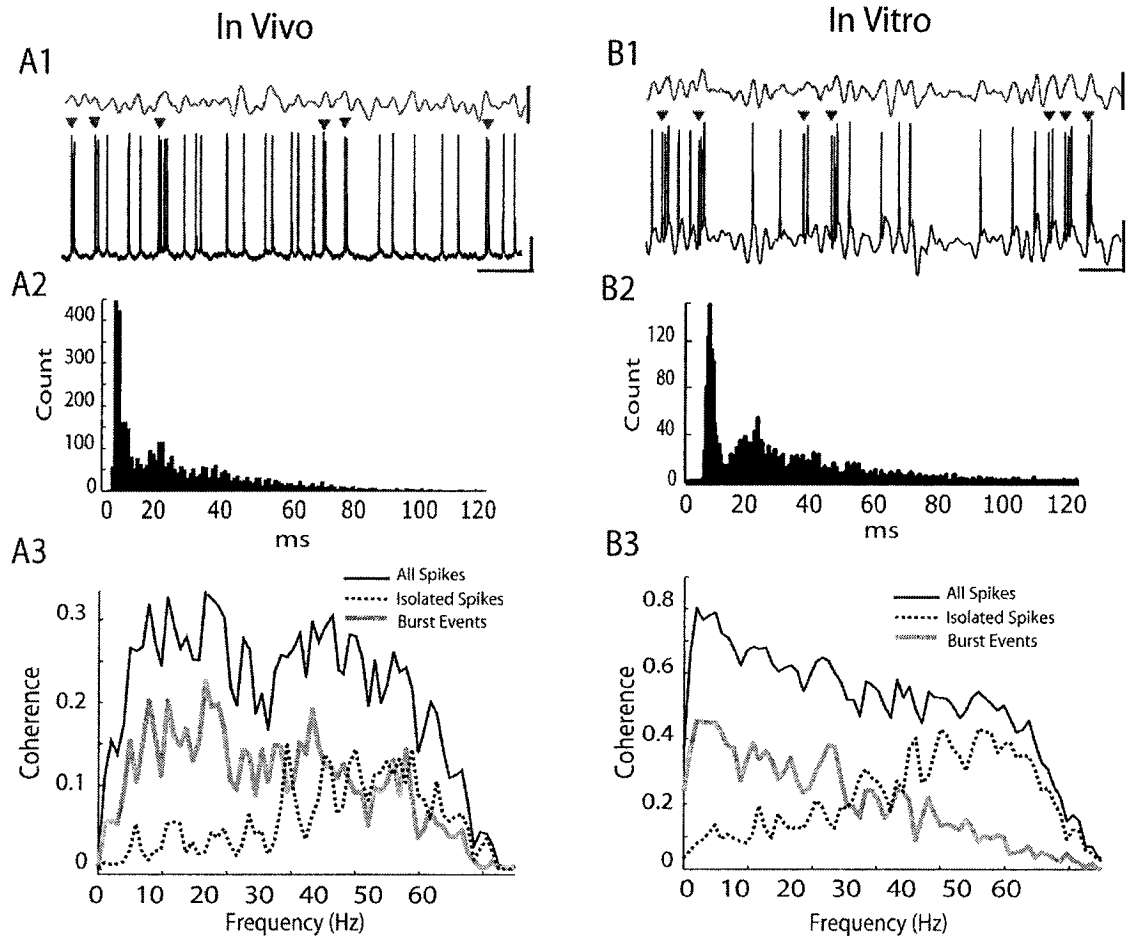


Figure 1: ELL Pyramidal cell responses to broadband (0-60 Hz) Gaussian noise

stimuli A) A pyramidal cell response *in vivo* to a localized application of the broadband stimulus at the skin; firing rate: 40 Hz, burst fraction: 0.49. B) A pyramidal response *in vitro* to direct current injection of the broadband stimulus; firing rate: 33 Hz, burst fraction: 0.27. A1, B1) Spike trains in response to broadband stimuli (grey) consisted of isolated spikes and high frequency bursts of spikes (arrowheads). A2,B2) Inter-spike interval (ISI) histograms with a significant peak in the 3-10 ms range that corresponds to the high frequency spike events. A3, B3) Coherence plots between the stimulus and the neural response for the full spike train (black, solid line) and the partitioned spike trains: burst events (thick grey line) and isolated spikes (dashed line). Scale bars in (A): Vertical: stimulus: 0.18 mV/cm, spike train: 20 mV; horizontal, 100ms. Scale bars in (B): Vertical: stimulus: 0.5 nA, spike train: 20 mV; horizontal: 100 ms.

The mean coherence values between 0-20 Hz (C_{low}), and 40-60 Hz (C_{high}) were calculated to assess the frequency selectivity of the event trains. These and other measured values are reported in Table 2 and the results of statistical analysis are presented in the text. Previous *in vivo* studies (Chacron, 2003) have demonstrated that pyramidal cells give low pass responses upon stimulation of their receptive field center and this was confirmed in the sample reported here ($C_{\text{low}} = 0.24 \pm 0.12$, $C_{\text{high}} = 0.10 \pm 0.097$, $p < 0.01$). Analysis of the *in vitro* full spike trains corroborated that the pyramidal cells were also low pass (*in vitro*- $C_{\text{low}} = 0.47 \pm 0.081$; $C_{\text{high}} = 0.308 \pm 0.08$, $p < 0.01$). The low pass response can be appreciated in the ratios of $C_{\text{low}} : C_{\text{high}}$, which were 2.4 and 1.5, *in vivo* and *in vitro* respectively.

Table 2: Summary of Measured Values *in vivo*, *in vitro* and the Model

		<i>In vivo</i>	<i>In vitro</i>				Model	
Stimulus		0-60 Hz	0-60 Hz	0-20 Hz	40-60 Hz	0-60 Hz		0-60 Hz
Condition						Control	TTX	ADM PDM
Firing Rate (sp/s)		22 ± 12.9	18 ± 4	16 ± 3	16 ± 2	18	18	32 Hz 33 Hz
Burst Fraction		0.49 ± 0.16	0.22 ± 0.12	0.32 ± 0.19	0.008 ± 0.005	0.16	0.08	0.24 0.064
n		33	24	6	6	3	3	n/a n/a
All Spikes	C_{low}	0.24 ± 0.12	0.47 ± 0.08	0.51 ± 0.09	n/a	0.5	0.5	0.67 0.72
	C_{high}	0.10 ± 0.10	0.31 ± 0.08	n/a	0.60 ± 0.10	0.32	0.34	0.38 0.39
Burst Events	C_{low}	0.13 ± 0.08	0.13 ± 0.07	0.15 ± 0.07	n/a	0.19	0.12	0.27 0.14
	C_{high}	0.03 ± 0.03	0.04 ± 0.02	n/a	0.02 ± 0.01	0.05	0.03	0.16 0.07
Isolated Spikes	C_{low}	0.08 ± 0.04	0.28 ± 0.08	0.42 ± 0.05	n/a	0.25	0.33	0.19 0.47
	C_{high}	0.08 ± 0.08	0.30 ± 0.14	n/a	0.54 ± 0.13	0.29	0.33	0.25 0.319
BTA	P_{low}	0.041 ± 0.055	0.20 ± 0.06					
	P_{high}	0.009 ± 0.054	0.05 ± 0.01					
STA	P_{low}	0.021 ± 0.054	0.06 ± 0.03					
	P_{high}	0.010 ± 0.016	0.04 ± 0.01					

Table 2: Summary of Measured Values *in vivo*, *in vitro* and the Model. Stimuli were Gaussian noise in the ranges 0-60 Hz, 0-20 Hz and 40-60 Hz applied *in vivo*, *in vitro*, or in the model as indicated. The mean firing rate and burst fraction during stimulation are reported. In some cases the stimulus was applied in the presence or absence of tetrodotoxin (TTX) *in vitro*, or in the presence of active dendritic backpropagation (active dendrite model, ADM) or the absence of backpropagation (passive dendrite model, PDM). Coherence between the stimuli and the full spike trains, burst trains and the isolated spike trains were measured. C_{low} and C_{high} correspond to the mean values of the coherence measured in the 0-20 Hz and 40-60 Hz ranges respectively. The power spectrums of the burst triggered average (BTA) and the spike triggered average (STA) were obtained. P_{low} and P_{high} correspond to the mean power measured in the 0-20 Hz and 40-60 Hz ranges respectively. All values are reported as mean +/- standard deviation; n/a, not applicable.

Bursts and isolated spikes code for different signal components

Pyramidal cells were capable of responding to the entire stimulus frequency range with bursts or isolated spikes. However, both *in vivo* and *in vitro*, burst responses were significantly biased toward low frequency stimulus events ($C_{low} > C_{high}$: *in vivo* and *in vitro*: $p < 0.01$) as the $C_{low} : C_{high}$ ratio *in vivo* and *in vitro* were 4.7 and 3 respectively. In contrast, isolated spikes are on average less selective ($C_{low} \approx C_{high}$: *in vivo*: $p > 0.5$; *in vitro*: $p > 0.5$) with $C_{low} : C_{high}$ ratios of 0.7-1.0. We further investigated the frequency selectivity of bursts by injecting low frequency (LF: 0-20 Hz) or high frequency (HF: 40-60 Hz) noise *in vitro* (Figure 2 A, B, respectively). During LF stimulation pyramidal cells responded with both bursts (2 ± 1 bursts/s, burst fraction= 0.32 ± 0.19) and isolated spikes (10 ± 3 spikes/s) while HF stimulation yielded almost exclusively isolated spikes (16 ± 2 Hz, burst fraction = 0.008 ± 0.005). These differential responses are apparent in the ISI histograms where LF stimulation resulted in bursts (Figure 2 A1- arrowheads) consisting of 2-8 spikes corresponding to a peak at 3-10 ms (Figure 2 A2) that were absent during HF stimulation (arrow, Figure 2 B2). During LF stimulation both bursts and isolated

spikes were coherent with the stimulus, though isolated spikes had higher coherence values (Bursts: $C_{\text{low}} = 0.15 \pm 0.07$; Isolated Spikes: $C_{\text{low}} = 0.42 \pm 0.05$, Figure 2 A3). In addition, isolated spikes were coherent with the HF stimulus ($C_{\text{High}} = 0.54 \pm 0.13$, Figure 2 B3). These results show that the isolated spikes can be coherent with either LF or HF stimuli and are therefore capable of stimulus estimation over the entire frequency range. Although bursts are selectively elicited by LF stimuli, the lower burst rate results in lower coherence values than those of isolated spikes. Thus, even at low frequencies, isolated spikes outperform bursts for stimulus estimation.

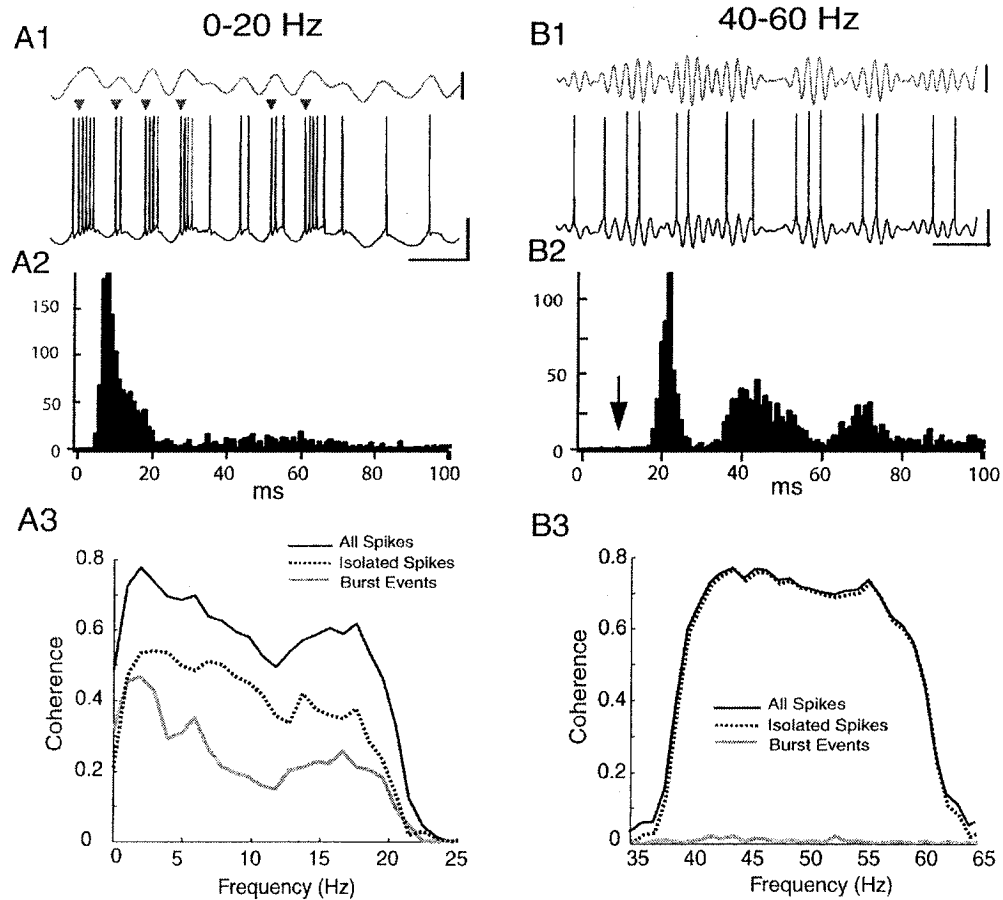


Figure 2: Pyramidal Cell responses *in vitro* to low frequency (0-20 Hz) versus high frequency (40-60 Hz) Gaussian noise stimuli. A1) The pyramidal cell response (black) to direct

current injection of a 0-20 Hz noise stimulus (grey) consisted of single spikes and high frequency bursts of 2-8 spikes (arrowheads). Firing rate: 20 Hz, burst fraction 0.49. A2) ISI histogram is strongly skewed toward short, 3-10 ms ISIs. A3) Coherence between the low frequency stimulus and the neural response for the full spike train (black, solid line) and the partitioned spike trains: burst events (thick grey line) and isolated spikes (dashed line). B1) The pyramidal cell response (black) to direct current injection of a 40-60 Hz noise stimulus (grey) consisted mainly of single spikes. Firing rate: 20 Hz, burst fraction 0.00. B2) The ISI histogram is multimodal, but lacks short, 3-10 ms ISIs (arrow). B3) Coherence between the high frequency stimulus and the neural response for the full spike train (black, solid line) and the partitioned spike trains: burst events (thick grey line) and isolated spikes (dashed line). Scale bars-Vertical: stimulus: 0.5 nA, spike train: 20 mV, horizontal: 100 ms.

In order to characterize the signals that evoke bursts versus single spikes, we computed the burst and spike triggered averages (BTA and STA, Figure 3 A).

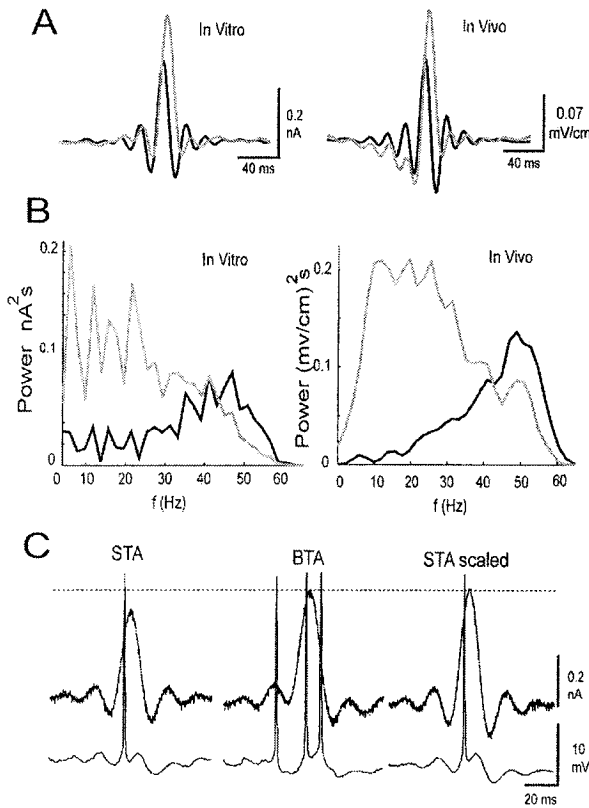


Figure 3: Burst and spike triggered averages are distinct stimulus features A) Burst (grey) and spike (black) triggered averages (BTA/STA) *in vitro* (left) and *in vivo* (right) were of significantly different height and duration (generated based on the responses to 0-60 Hz stimulation of the cells presented in Figure 1). B) The power spectrum of the BTA (grey) was significantly low pass and that of the STA (black) was broadband to high pass both *in vitro* and *in vivo*. C) Re-injection of the STA or BTA *in vitro* produced single spikes and doublets respectively. Scaling the STA to the amplitude of the BTA did not elicit doublets in the majority of presentations.

Both *in vivo* and *in vitro*, the power spectra of the BTA and STA are qualitatively similar to the coherence curves for burst events and isolated spikes respectively (Figure 3 B): the BTA has power (P) predominantly at low frequencies ($P_{\text{low}} > P_{\text{high}}$; *in vivo*: $p = 6.8 \times 10^{-4}$; *in vitro*: $p = 1 \times 10^{-9}$) while the STA has significant power over the entire frequency range ($P_{\text{low}} \approx P_{\text{high}}$; *in vivo*: $p > 0.1$; *in vitro*: $p > 0.1$).

We then explored the dependence of bursts on the amplitude and temporal characteristics of the BTA *in vitro*. As expected, re-injection of BTA and STA waveforms resulted in bursts (81 ± 8.5 % of presentations) and spikes ($94 \pm 2\%$) respectively (Figure 3 C). The BTA was of significantly greater amplitude ($p < 0.01$) and half-width ($p < 0.01$) than the STA. In some systems the BTA and STA are scaled versions of each other (Gabbiani, 1996; Reinagel, 1999) suggesting that increasing the stimulus amplitude would be sufficient to induce bursting. However, scaling the STA to the height of the BTA produced bursts in only $10 \pm 3\%$ of stimulus presentations. Alternatively, we doubled the standard deviation of the 0-60 Hz stimulus. The spectral properties of the BTA and STA were conserved (data not shown) but the amplitude of the STA was increased to 0.6 nA, a value that surpasses the amplitude of the BTA under control conditions (0.45 nA). This very strong stimulus increased low frequency burst coherence but had a minimal effect on burst coherence with high frequencies ($\Delta C_{\text{high}} = 0.037 \pm 0.002$; $\Delta C_{\text{low}} > \Delta C_{\text{high}}$; $p < 0.01$, $n=14$). These results demonstrate that bursting is dependent on the temporal characteristics of the stimulus as well as its amplitude; this conclusion is consistent with theoretical studies of electrosensory pyramidal cell burst dynamics (Laing, 2003).

Bursts in ELL pyramidal cells therefore appear to selectively signal low frequency components of sensory input, while isolated spikes are able to encode the entire frequency range. The bursts analyzed above were empirically defined from the ISI histogram and we next explore their relation to the dynamically defined ghostburst mechanism.

Backpropagation Dependent Bursting

ELL pyramidal cells have a well-characterized burst mechanism that is dependent on the active backpropagation of somatic spikes along the proximal apical dendrite (Lemon, 2000; Doiron, 2001, 2002). We used constant current injections (0.5-1.0 nA) *in vitro* to verify that backpropagation dependent bursting can be elicited in all neurons studied. Figure 4 shows dendritic (A) and somatic (B) recordings *in vitro* in response to a constant depolarizing current injection.

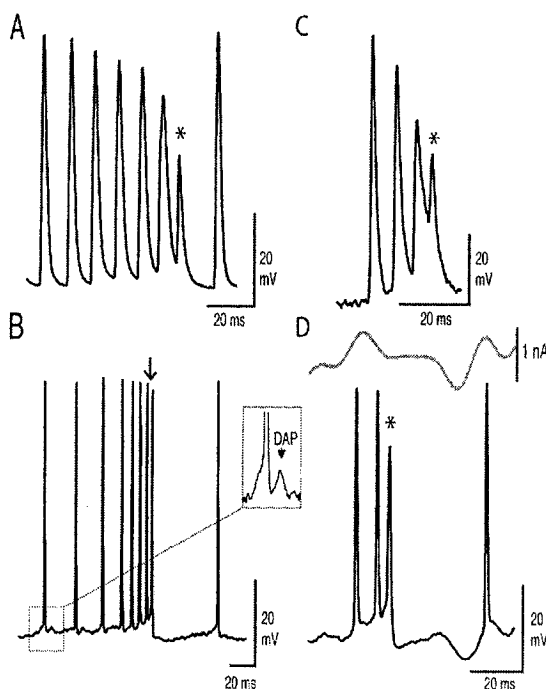


Figure 4: Evidence for backpropagation dependent bursting *in vitro* and *in vivo*. A) Dendritic recording of a failure (*) of spike backpropagation elicited by step depolarization *in vitro*. B) Somatic recording of a backpropagation dependent burst elicited by step current *in vitro*. Note the depolarizing after potential (DAP) due to dendritic spike (inset) that leads to high frequency doublet generation (arrow). C) Dendritic failure in response to a step change in the EOD amplitude *in vivo*. D) Dendritic failure in response to broadband noise stimulation (grey) *in vitro*.

Somatic spikes backpropagate along the dendrite and broaden as the burst evolves. This is reflected at the soma as an increasing depolarizing after potential (DAP) (inset, Figure 4 B). Finally, DAP potentiation reaches the threshold for high frequency doublet generation (arrow, Figure 4 B). However, the doublet ISI falls within the dendritic spike refractory period halting the backpropagation of the second action potential of the doublet (asterisk, Figure 4 A), consequently terminating the burst. The doublet ISIs constitute a substantial peak in the 3-10 ms range of the ISI histogram (Turner, 1994; Doiron, 2003). In addition, 0-60 Hz stimulation *in vitro* yields ISI histograms with a prominent peak in the 3-10 ms range (Figure 1 B2) and results in dendritic failures (Figure 4 D). Likewise, ISI histograms with a peak in a similar time range are observed in response to 0-60 Hz stimulation of a subset of pyramidal cells with high spontaneous firing frequencies (Figure 1 A2). Bursting and dendritic failure can also be elicited by step increases in EOD amplitude in some pyramidal cells *in vivo* (Figure 4 C). Thus, we propose that the high frequency doublets present during 0-60 Hz stimulation are due to the backpropagation-dependent burst mechanism *in vitro*. This same mechanism is likely to underlie bursting in at least a subset of cells *in vivo*, however, additional studies are needed to determine if the same mechanism gives rise to bursting in pyramidal cells with lower spontaneous firing frequencies (Bastian and Nguyenkim, 2001).

It has been demonstrated that the backpropagation of somatic action potentials along the dendrite is dependent on voltage dependent sodium (Na^+) conductances (Turner, 1994; Doiron, 2003). We focally applied Tetrodotoxin (TTX) to the proximal apical dendrite of pyramidal cells *in vitro*. As previously demonstrated (Turner, 1994) this eliminated bursting induced by constant current (data not shown). TTX application

also greatly reduced the number of high frequency doublets elicited during 0-60 Hz noise injection (Figure 5 A) and the ISI histogram became unimodal (Figure 5 B); it should be noted that the average firing rate did not change significantly. These results show that the bursts seen during broadband *in vitro* current injection are due to active dendritic processes.

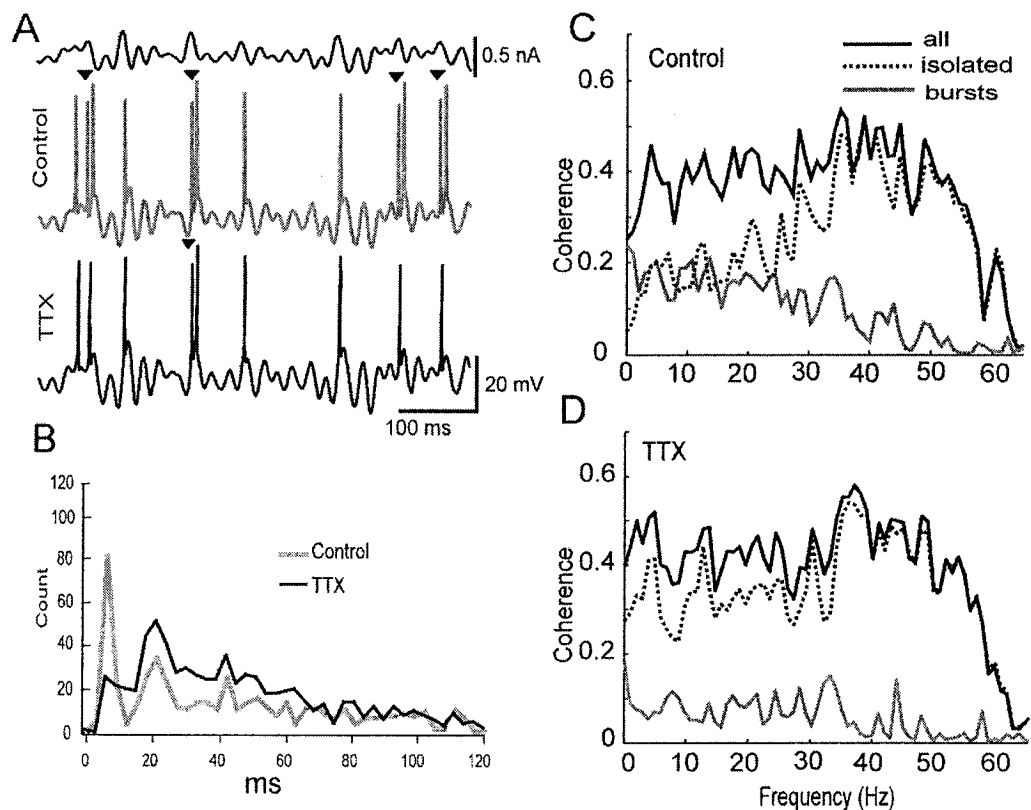


Figure 5: TTX blocks bursting but does not affect overall low frequency coherence.

A) Pyramidal cell response to a broadband stimulus (top trace) before (grey) and after (black) application of TTX to the apical dendrite. The number of bursts (arrows) is greatly reduced by TTX, which is reflected in the decreased peak in the 3-10 ms range of the ISI histogram (B), control (grey), TTX (black). C) Coherence of bursts (grey), isolated spikes (black-dashed) and all spikes (black-solid) with the stimulus prior to application of TTX. D) In the presence of TTX, burst coherence decreased (grey) while isolated spike coherence (black-dashed) increased. The coherence of the full spike train did not change significantly (black-solid).

Since bursts are low frequency coherent, we hypothesized that preventing bursts during broadband current injection would decrease the low frequency coherence of the full spike train. During TTX application, there was an expected reduction in burst coherence that coincided with an increase in low frequency coherence for isolated spikes (Figure 5 C, D). However, surprisingly, there was not a substantial change in full spike train coherence (Figure 5 C, D; $n=3$). Contrary to our hypothesis, these results suggest that only the first spike of a burst is important for low frequency coherence and that bursts do not appear to code additional information about the stimulus.

Single spikes effectively code low frequency stimuli

TTX applied to the apical dendrites eventually diffuses to the soma and blocks spiking; thus, there was only a limited time for data acquisition during which recordings were stationary and the somatic Na^+ was not affected. We therefore further investigated the role of backpropagation-dependent bursts in stimulus encoding using a two-compartment reduction of the detailed ionic model of an ELL pyramidal cell (Doiron, 2002). The model parameters and stimulation protocols (see Methods) were adapted so that the firing rate and burst fractions reported for the model were comparable to those presented *in vitro* (Figure 1 B).

Injection of 0-60 Hz Gaussian noise into the model neuron yielded similar results to those seen *in vivo* and *in vitro*. Spike trains at the soma contained both single spikes and bursts (arrowheads) (Figure 6 A1). The majority of bursts were high frequency doublets, which corresponded to failures in the dendrite (Figure 6 A1, asterisks). The ISI histogram was bimodal with peaks at 3-5 ms and 20 ms (Figure 6 B, thin grey line). An ISI criterion of 6 ms, which corresponds to the trough of the ISI histogram was chosen to

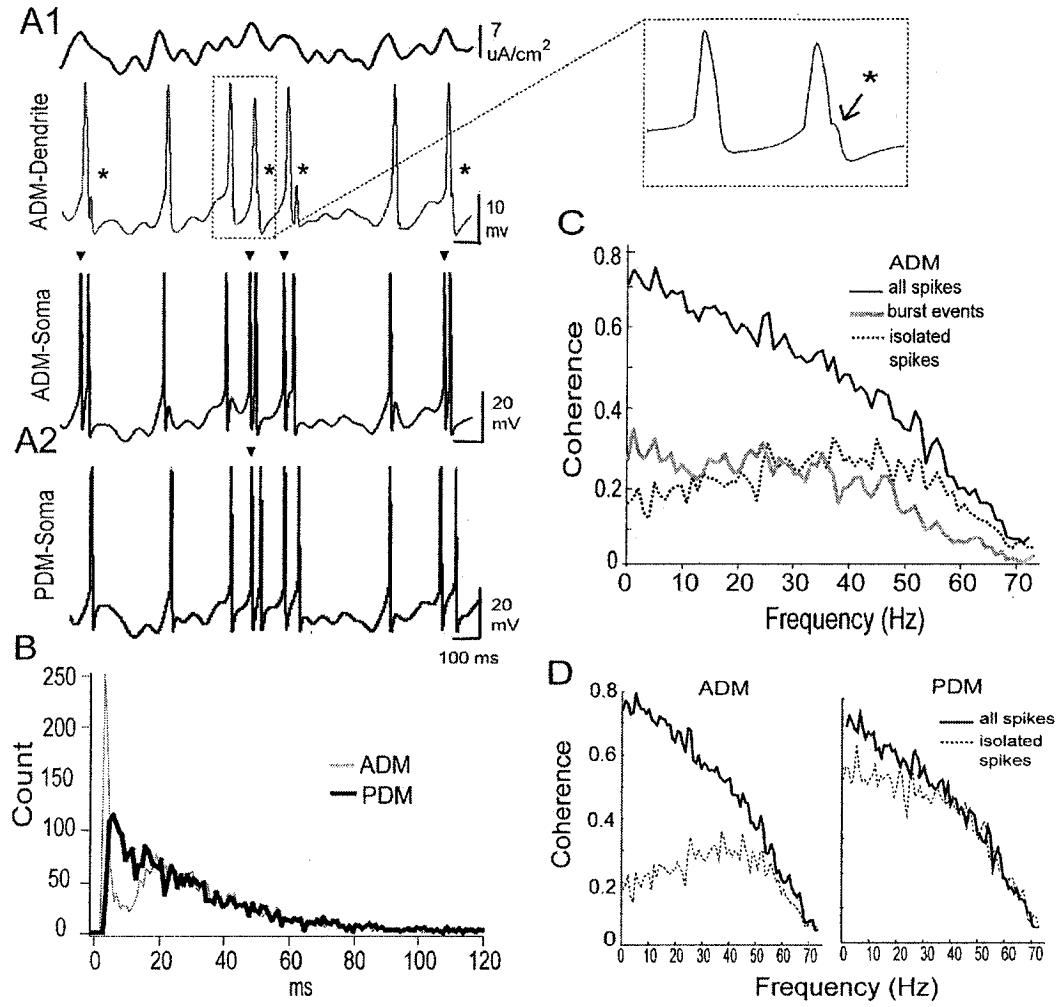


Figure 6: The model pyramidal cell recreates *in vitro* responses to broadband

stimuli A) Model response to injection of the broadband stimulus (top trace). Two cases are presented one in which the model neuron has an active dendrite (ADM) (A1) and one in which the dendritic Na^+ conductance is set to zero (passive dendrite model, PDM) (A2). The ADM had a firing rate of 32 Hz and somatic spike trains contained isolated spikes and high frequency bursts (arrowheads, burst fraction: 0.24) that corresponded to dendritic failures (asterisks). The PDM had a firing rate of 33 Hz but a burst fraction of 0.03. B) Inter-spike interval (ISI) histogram of the ADM (bimodal, thin grey line) with a significant peak in the 3-5 ms range that correspond to burst events and the PDM (unimodal, thick black line). C) Coherence plots between the stimulus and the ADM response for the full spike train (black-solid line) and the partitioned spike trains: burst events (thick grey line) and isolated spikes (black-dashed line). D) Coherence of the full spike train (solid line) and isolated spikes (dashed line) with the stimulus in the ADM (left) and PDM (right). Scale bars ($7 \mu\text{A}/\text{cm}^2$, 20 mV, 100 ms, unless otherwise noted).

distinguish burst spikes. Based on this criterion, the coherence between each of the event trains (all spikes, burst events, and isolated spikes) and the stimulus were calculated as described above. Similar to pyramidal cells *in vivo* and *in vitro*, the model neuron was low pass, and bursts were low frequency coherent while single spikes had a broadband response (See Table 2, Figure 6 C, compare with Figure 1).

We mimicked TTX blockade of active dendritic processes and prevented burst discharge by setting the dendritic, spiking Na^+ conductance ($g_{\text{max,Na,d}}$) to zero, effectively making the dendrite passive. From this point on, this will be referred to as the passive dendrite model (PDM) while the model with active dendritic processes intact will be referred to as the active dendrite model (ADM). The injection of a 0-60 Hz stimulus into the PDM results in an ISI histogram that still contains high frequency ISIs (8-10 ms), but the bimodality and the highest frequency doublet ISIs (< 6ms) are lost (Figure 6 A2, B, thick black line). As with experimental blockade, there was a large reduction in burst fraction and low frequency burst coherence (Table 2, data not shown); because burst events are reduced to single spikes there is a substantial increase in the low frequency coherence of isolated spikes (Figure 6 D, dashed lines). Consistent with experimental data, there was no substantial change in the coherence of the full spike train (Figure 6 D solid lines). Thus, removing active backpropagation may result in a loss or delay of the second spike of the doublet, but this appears to be inconsequential because the majority of information appears to be coded in the first spike of a burst.

Bursts and isolated spikes implement different coding strategies

While single spikes provide better estimates of the detailed time course of the stimulus, it has been suggested that bursts may be more reliable indicators of specific

stimulus features (Lisman, 1997). We employed the Fisher linear discriminant (Gabbiani, 1996; Metzner, 1998) to evaluate the effectiveness of feature detection by burst events versus single spikes. Burst events were distinguished from single spikes based on the previously described ISI criteria and the following events were identified: all spikes (bursts + isolated spikes), burst events, isolated spikes, and the null distribution (no bursts or isolated spikes). Stimulus features that corresponded to the 50 ms stimulus epoch that preceded each event were identified and then grouped into distributions according to the event elicited. An optimal feature was determined for each event distribution that maximized the separation between that event distribution and the null distribution. Each individual stimulus feature was then compared to the optimal feature and, based on the degree of similarity, a prediction was made as to the whether or not that event (single spike, burst or null) will occur. The probability of correctly predicting an event versus the probability of false alarm is plotted as the receiver operating characteristic (ROC) curve.

Figure 7 shows the feature extraction of bursts versus single spikes in response to 0-60 Hz stimulation in representative pyramidal cells, *in vivo* (Figure 7 A) and *in vitro* (Figure 7 B) as well as to 0-20 Hz and 40-60 Hz stimulation *in vitro* (Figure 7 C, D). The optimal features (solid lines) detected by all spikes (black) bursts (thick grey) and isolated spikes (dashed) in response to each stimulus are presented in Figure 7 A2, B2, C2 and D2. The distributions arising from the projection of the stimulus features onto the optimal features are shown in Figure 7 A3, B3, C3 and D3; a reduced overlap between the event distributions (bursts-grey, isolated spikes-dashed) and the null distribution (black line) indicates better feature detection.

In response to 0-60 Hz stimulation both *in vivo* and *in vitro*, bursts have higher SNR values (Figure 7 A1, B1 insets,) than the full ($\text{SNR}_B > \text{SNR}_F$; $p < 0.01$) and isolated ($\text{SNR}_B > \text{SNR}_I$; $p < 0.01$) spike trains. In addition, isolated spikes have lower SNR values than the full spike train ($\text{SNR}_F > \text{SNR}_I$; $p < 0.01$). The SNRs *in vitro* are much higher than *in vivo* due to the much lower rate of spontaneous activity *in vitro* compared to *in vivo*. Comparable results to *in vitro* responses were also obtained for the same stimulus in the model neuron (data not shown). The optimal features are derived from the BTA (low pass) and STA (broadband), which differ in their frequency content (Figure 3). In addition, bursts are coherent with the low frequency content of the stimulus. For these reasons, we propose that bursts are detectors of low frequency stimulus features. The situation for isolated spikes is more complex (see below).

Because the optimal features detected by bursts and isolated spikes during 0-60 Hz stimulation differ, we cannot directly assess the discrimination performance of bursts or single spikes when they are coding similar frequency ranges. Thus, we directly compared the feature detection capability of bursts and isolated spikes for narrow bands of low (LF: 0-20 Hz) and high frequency (HF: 40-60Hz) stimuli. In response to LF stimuli, bursts have better signal to noise ratios (SNR) than isolated spikes (SNR_B : 3.76 ± 0.23 ; SNR_I : 2.36 ± 0.26 ; $p < 0.05$) (Figure 7 C1, inset). The optimal feature detected by bursts is a scaled version of the isolated spike feature with the burst feature having a higher amplitude threshold for bursting (Figure 7 C2). Thus, bursts are more selective for their stimulus features than single spikes. Conversely, the lower threshold for isolated spikes leads to tonic firing that tracks the stimulus whenever the membrane potential is above threshold. This improves coherence and therefore stimulus estimation (see Figure

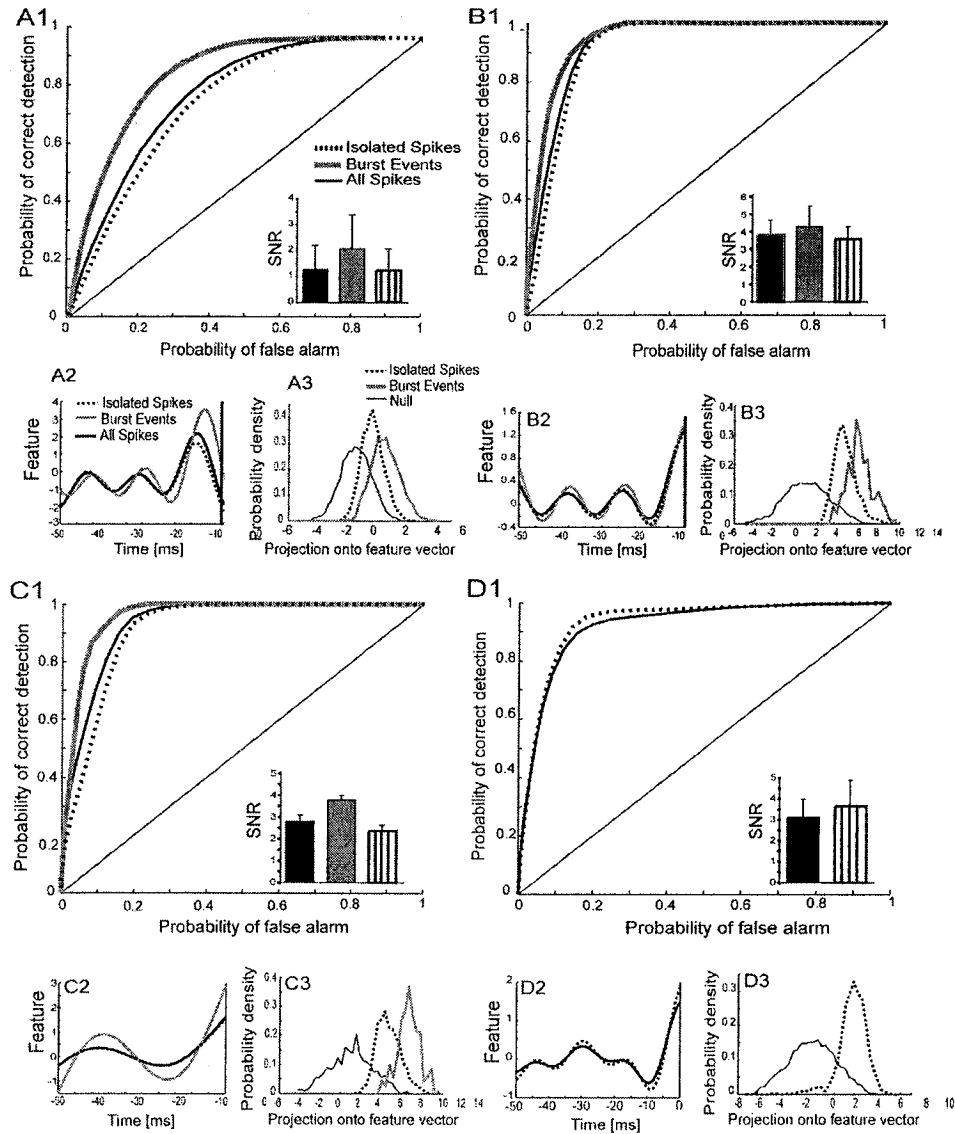


Figure 7: Feature extraction *in vivo* and *in vitro* A) Analysis of spike trains in response to a broadband stimulus *in vivo*. B) Analysis of spike trains in response to a broadband stimulus *in vitro*. C) Analysis of spike trains in response to LF stimulation (0-20 Hz) *in vitro*. D) Analysis of spike trains in response to HF stimulation (40-60 Hz) *in vitro*. A1-D1) ROC curves for the partitioned spike trains; all spikes (black-solid), bursts (thick grey) and isolated spikes (black-dashed). Inset: the mean SNR of all spikes (black-solid), bursts (grey) and isolated spikes (black striped). A2-D2) The optimal features detected by all spikes (black-solid) bursts (grey), and isolated spikes (black-dashed). Note that extended structure of these features (<-20 ms) may be due to the narrow-band *Nature* of the stimulus set. This may induce long-range correlations in the stimulus waveforms that might not be causally related to spikes and not eliminated by spike-triggered averaging. A3-D3) Projection onto the optimal features, bursts (grey), isolated spikes (black-dashed) and the null feature (black-thin).

2) but reduces the difference between the optimal feature and the null feature, which hinders feature detection.

Alternatively, when a HF stimulus (40-60 Hz) is presented, isolated spikes have high SNR values (Figure 7 D1, inset). Though the lack of bursts prevents a statistical comparison between isolated spikes and bursts; in 4 of 6 cells the SNR for isolated spikes was greater than for the full spike train, suggesting that rare instances of bursting may actually compromise high frequency stimulus detection.

These results demonstrate that bursts, though not necessary for stimulus estimation, offer a significant advantage over isolated spikes for low frequency feature detection. Conversely, isolated spikes are better estimators of low frequency stimuli. At high frequencies, isolated spikes could be considered as either feature detectors or stimulus estimators depending on the downstream decoding mechanism.

Burst Dynamics are required for feature detection

The implication that bursts are more reliable feature detectors than isolated spikes is based on the hypothesis that sequential activation at a synapse by two or more spikes separated by short ISIs is more effective than when the spikes are separated by long ISIs (Lisman, 1997). This raises the question of whether short ISIs that are a consequence of a biophysical burst mechanism provide an advantage for information transfer as compared to short ISIs that are not the result of a burst mechanism. We again used the ghostbuster model to investigate whether bursting dynamics are required to generate high frequency multi-spike events that are selective for low frequencies. In Figure 8, three scenarios are presented in which we attempted to recreate the low-frequency selectivity and the feature extraction in the model neuron with a passive dendrite (PDM) to match that of the active dendrite model (ADM). As previously shown, removing burst

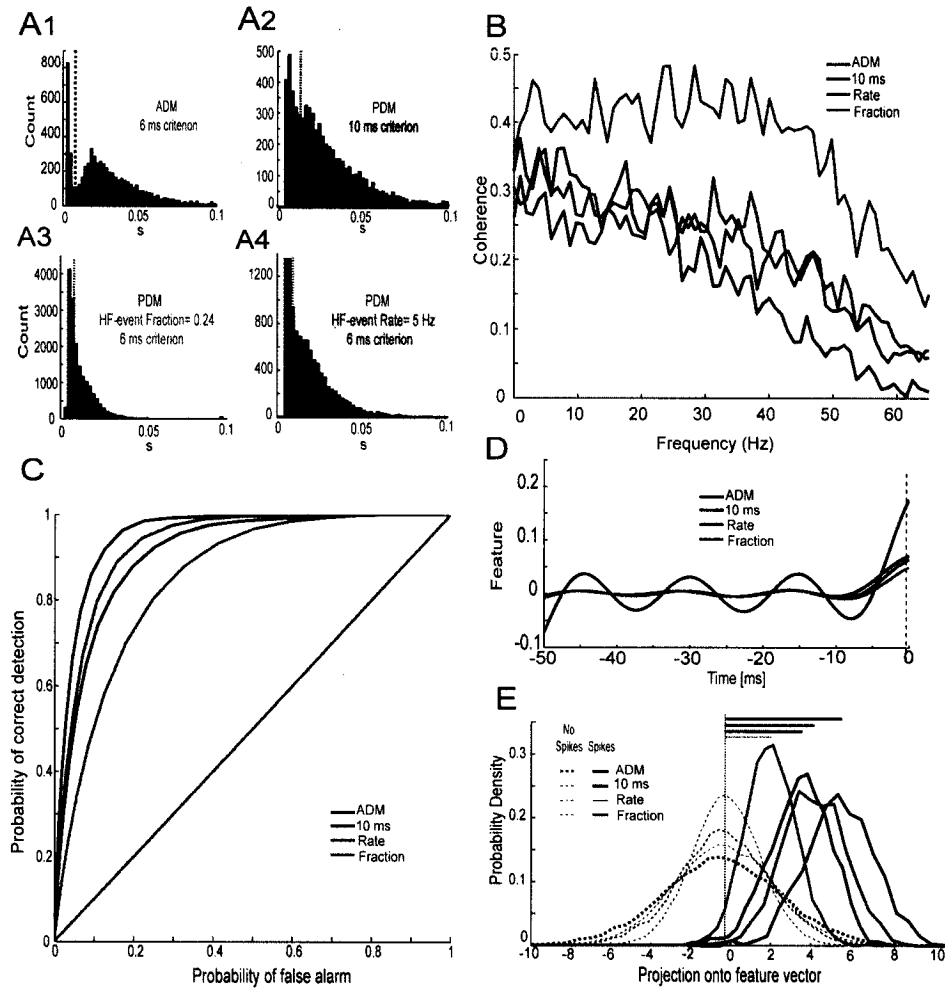


Figure 8: Burst dynamics are required for feature detection. A) ISI histograms in response to broadband stimulation of the model neuron with an active dendrite (ADM) or a passive dendrite (PDM). A1) Bimodal histogram of the ADM, dashed black line indicates the 6 ms ISI criterion for bursts (see *Methods*), firing rate: 33 Hz. A2) Unimodal histogram of the PDM, blue line indicates 10 ms ISI criterion that conserves the fraction (0.24) and rate (5 Hz) of high frequency (HF) events seen in the ADM, firing rate: 33 Hz. A3) PDM histogram in response to an increase in I_0 that increased the firing rate to 80 Hz but conserved the *fraction* of <6 ms HF-events generated by the ADM (red line). A4) PDM histogram in response to an increase in I_0 that increased the firing rate to 50 Hz and conserved the *rate* of <6 ms HF-events generated by the ADM (green line). B) Burst (ADM) or HF-event (PDM) coherence with the broadband stimulus (colors as determine and indicated in A). C) ROC curves of bursts (ADM, black) or HF-events (PDM, colors as in A). D) Optimal features detected bursts or HF-events in each scenario. E) Feature projections in each scenario onto the optimal feature (solid lines) as compared to the null feature (dashed lines).

dynamics shifted the ISI histogram from a bimodal (Figure 8 A1) to unimodal distributions (Figure 8 A2-A4).

Since high frequency (HF) events are no longer well defined, we identified subsets of HF-events based on ISI criteria (colored vertical lines, Figure 8A2-4) that resulted in the same HF-event *rate* (5 Hz) or *fraction* (0.24) as indicated by the bimodal histogram of the ADM. In the first scenario, we changed the definition of a HF-event from an ISI < 6 ms to an ISI <10 ms such that the mean firing rate and the HF-event *rate and fraction* were the same as the ADM. The HF-events were low pass (Figure 8B, ADM (black), PDM-10 ms ISI (blue)) but were poor feature detectors compared to true ADM bursts (Figure 8C, Control (black), 10 ms ISI (blue)).

To ensure that reduced feature detection in the PDM was not due to the loss of ISIs less than 6 ms, in the second and third scenarios we increased the offset current, I_0 , injected into the PDM and used 6 ms criterion to distinguish HF-events (Figure 8 A3 & A4). In the second scenario the fraction of HF-events with ISIs<6 ms was comparable to the *burst fraction* in the ADM. The PDM required 18 times more current to generate the same fraction of ISIs < 6 ms as the ADM, which increased the mean firing rate to 80 Hz and the HF-event rate, to 15 Hz. This resulted in broadband, HF-event coherence (Borst, 2001) but very poor feature detection (Figure 8 B, C (red lines)).

In the final scenario the rate of ISI events <6 ms was comparable to the *burst rate* in the ADM. Again, in order to produce a HF event rate of 5 Hz, the passive neuron required 5 times the current resulting in a higher mean firing rate (50 Hz) than the ADM. These events were low frequency coherent but also failed to recover the same degree of feature extraction as true bursts (Figure 8 B, C (green lines)).

These results demonstrate that neurons with passive dendrites can generate HF-events (ISIs < 6 ms or <10 ms) that are coherent with low frequencies. However, in each instance, the optimal feature detected by the HF-event is of lower amplitude at the time of the event ($t=0$) compared to the ADM (black) (Figure 8D). This decreases the distinction between the distribution of features detected by HF-events and the null distribution. When stimulus features are projected onto these optimal features, there is less distinction between the HF-event distributions and the null distribution (Figure 8E) leading to a higher probability of false alarm. The end result is a reduced ability to detect specific stimulus features, even when the high frequency event rate is conserved. Thus, backpropagation dependent pyramidal cell bursts are not only low frequency selective, but the burst mechanism enables pyramidal cells to generate a distinct stream of high frequency burst events that demonstrate superior feature extraction capability over non-burst HF-events.

Discussion

We have demonstrated, both *in vivo* and *in vitro*, that in response to broadband stimuli, ELL pyramidal cells generate a spike train that consists of two parallel information streams. Specifically, ELL pyramidal cells selectively detect low frequency features of broadband Gaussian stimuli with spike bursts while single spikes simultaneously estimate the entire stimulus frequency range.

Interestingly, the low frequency coherence of pyramidal cell responses was not dependent on burst dynamics as the coherence between full spike train and the stimulus was unaffected by the dendritic application of TTX *in vitro* and by removing dendritic Na^+ conductance in the ghostbuster model. Under these conditions, a low frequency

event triggers a somatic spike, which is coherent with the stimulus. However, this spike fails to backpropagate and additional burst spikes do not occur. This demonstrates that the low frequency coherence with the stimulus is carried in the timing of the first spike of the burst (Kepecs, 2003). Since coherence is directly related to the linear estimation of a signal from a spike train (Gabbiani, 1998; Roddey, 2000), we conclude that bursts are not necessary for this estimate.

We quantified the selectivity of bursting neurons using a previously described feature extraction technique (Metzner, 1991; Gabbiani, 1996). Although single spikes are sufficient to estimate low frequency input, our *in vitro* results demonstrate that bursts are substantially better detectors of these signals. While this corroborates previous *in vivo* results (Metzner, 1991; Gabbiani, 1996), we expand on these studies by showing that feature detection by bursts is low frequency specific and, at least in some cells, dependent on the backpropagation dependent burst mechanism.

Benefits of Burst Dynamics in Neural Coding

The analysis of feature extraction by the model neuron with intact burst dynamics revealed that bursts were superior feature detectors even when compared to similar high frequency spike events produced by the model neuron with a passive dendrite. Moreover, the passive dendrite model required substantially higher input current than the bursting model neuron to generate comparable numbers of high frequency spike sequences, which significantly increased the mean firing rate. Thus, while coherence and stimulus estimation are enhanced by increases in firing rate (Borst and Haag, 2001), the reduced feature selectivity of the non-bursting neuron shows that these high frequency events cannot adequately substitute for bursts when they are used as feature detectors.

Furthermore, it has been argued that neural codes should be sparse and implement low firing rates in order to be efficient (Rolls, 1995; Vinje, 2000). Burst dynamics may allow for such codes since they permit low firing rates while having a significant proportion of distinct high-frequency burst events. We therefore propose that pyramidal cells may use high-frequency bursts to selectively detect and efficiently code for the low-frequency components of stimuli.

Decoding Pyramidal Cell Output

For bursts to be an important component of a neural code requires an appropriate postsynaptic decoding mechanism. For instance, a presynaptic burst ISI may match a resonance timescale of a postsynaptic neuron, and gate information transfer (Izhikevich, 2003). Alternatively, bursts in conjunction with short term synaptic plasticity could improve information transfer at unreliable synapses (Lisman, 1997). ELL pyramidal cell output is decoded in the torus semicircularis (TS) of the midbrain and both synaptic depression and facilitation may contribute to the selective extraction of low frequency features. Fortune and Rose (1997) have demonstrated that different TS cells in a related electric fish are tuned for various stimulus frequency ranges (low pass, broadband and high pass). The output of the TS is conveyed to brain regions selectively responsible for electrolocation (low frequency, optic tectum) or electrocommunication (low to high frequencies, nucleus electrosensorius) (Bell, 2004). A number of TS neurons temporally filter electrosensory stimuli resulting in low-pass (3-8 Hz), broadband (0-30 Hz), and high-pass cells (>10 Hz) (Fortune, 1997). In the case of low-pass cells, responses to stimulus frequencies greater than 10 Hz are attenuated by synaptic depression. Interestingly, this depression can be overcome since these cells also show marked

facilitation to high-frequency (~10 ms ISI) presynaptic activity (Fortune, 2001). In this study, we have shown that ELL pyramidal cells convert low-frequency sensory input to high-frequency bursts. Thus these bursts provide the high-frequency events that can be reliably detected by TS low-pass neurons. ELL pyramidal cells can therefore simultaneously code for both low-frequency stimulus components, via bursts, as well as high frequency components, via isolated spikes. Different TS cell populations (low-, broadband, or high-pass) can then selectively decode information related to low frequency signals emanating from prey versus high-frequency communication signals.

The ELL and the Thalamus as Sensory Gateways

The parallels between the ELL-Toral connections and thalamocortical pathways have been recently reviewed (Berman, 1999; Krahe, 2004). Thalamic bursting has been both biophysically characterized and modeled (Lu, 1992; McCormick, 1992; Mukherjee, 1995; Smith, 2000). In lateral geniculate (LGN) relay cells, bursts have been shown to exhibit frequency tuning (Mukherjee, 1995; Smith, 2000; Fortune, 2001) and enhance detectability at stimulus onset (Sherman, 2001; Martinez-Conde, 2002). Furthermore it has been suggested that depending on the specific LGN-cortical layer projection, bursts and single spikes could present distinct streams of information transfer for the *same* stimulus features (Reinagel, 1999) and that feedback from the cortex could switch coding modes between signal detection and stimulus estimation (Sherman, 2001).

Likewise, ELL pyramidal cell bursting is regulated through several dendritic and somatic ionic conductances (Noonan, 2003) and could conceivably be modulated by feedback input to the apical dendrites (Berman and Maler, 1999; Bastian and Nguyenkim, 2001). Recent studies have shown that ELL pyramidal cell responses to sensory input

depend on both the frequency content and spatial extent of the stimulus (Reinagel, 1999; Bastian, 2002; Chacron, 2003; Doiron, 2003) and that feedback is differentially activated by spatially localized versus spatially extended stimuli (Doiron, 2003; Bastian, 2004; Doiron, 2004). Thus, not only could feedback to the ELL switch coding strategies from feature detection by bursts to stimulus estimation by single spikes as proposed for the thalamus, but could also lead to the differential coding of distinct behaviorally relevant low (prey like) versus high (communication) frequency stimuli.

Chapter 2

Stimulus Dependent Spike Timing in Electrosensory Pyramidal Cells

Anne-Marie Oswald

Unpublished Results

Introduction

In the previous chapter, the coherence measure was used to show that the occurrence of burst and isolated spike events is correlated with low and broadband stimulus frequencies respectively. Coherence is a relative measure that quantifies the degree to which the frequencies of spike train events (spikes or bursts) is correlated with the frequencies of the stimulus. The results do not provide information about the exact timing of spike train events with regard to individual stimulus events. Nevertheless, analysis of the burst and spike triggered averages revealed that spike train events generally correspond to a single stimulus event consisting of an upstroke and a down stroke. It is conceivable that the precise timing of a spike or burst during an upstroke may be an important aspect of the neural code. This will be discussed in the present chapter.

In order for spike timing to be a robust aspect of the neural code, the timing of spike events should be both reliable and precise. An event is considered reliable if it occurs at approximately the same time each time a given stimulus is presented. The precision of an event is a measure of the variability in the spike times for that event over all stimulus presentations. Reliable spike timing has been demonstrated in a number of different cell types including cortical pyramidal cells and interneurons (Mainen, 1995; Fellous, 2001; Schreiber, 2004) and thalamic neurons (Reinagel, 2002). Reproducible spike train responses have also been elicited from electrosensory afferents (Kreiman, 2000) and pyramidal cells (Chacron, 2003). However, these studies did not directly measure the reliability and precision of pyramidal cell responses.

In this chapter, I demonstrate that the timing of ELL pyramidal cell spike train events in response to repeated presentations of comparable broadband inputs (similar to those presented in the previous chapter) is both reliable and precise. In addition, I investigate the role of precise spike timing with regard to the generation of burst events and discuss how interactions between spike timing and the stimulus time course enable bursts to be better indicators of stimulus upstrokes than isolated spikes.

Methods

Stimulation: ELL pyramidal cells ($n=20$) were stimulated with repeated presentations of shortened versions (3-10 s) of the 0-60 Hz, Gaussian noise stimulus described in the previous section. The standard deviation of the stimulus was varied from 0.17-2.0 nA. Shorter stimuli were presented more often (30-50 trials) than longer stimuli (10-20 trials).

Analysis-All Spikes: The measurements of reliability and precision are adaptations of the techniques developed by Mainen and Sejnowski (1995) and Berry and Meister (1998).

Reliability: The resulting spike trains were binned in 0.5 ms intervals, such that no more than one spike occurred per bin and an event histogram (similar to the PSTH) was constructed. Due to the frequency content of the stimulus, there is typically only one spike or burst per upstroke. This resulted in 10-16 ms periods of quiescence that, in combination with the narrow bin-width facilitated the identification of single spike train events. An event was determined as consecutive non-zero bins in the event histogram. These consecutive bins were summed and if the resulting spike number was greater than 66% of the number of trials, the spikes within that event were considered reliable. The reliability of the cell was determined as the number of reliable spikes divided by the total number of spikes occurring over all trials.

Precision: The histograms corresponding to reliable events were then fit with Gaussians. The width of the Gaussians provided a measure of the precision of each event. The precision of the cell was the average precision over all reliable events.

Analysis-Bursts: The reliability and precision of burst events was determined as described in the previous section, with the timing of the burst event corresponding to that of the first spike of the burst. In addition, the timing of burst and isolated spike events with respect to events in the stimulus was assessed. In order to acquire a sufficient number of burst events for analysis, the neural responses to longer (100 s) broadband stimuli that produced high proportions of bursts were analyzed. The time of burst or isolated spikes was subtracted from the time of the peak of the nearest upstroke. If the result was positive, the event occurred on the upstroke. Negative values correspond to occurrences on the down stroke. Values near zero indicate that the event occurred at or near the peak of the upstroke.

Results

Analysis of All Spikes

The raster plots of spike times for each trial in response to repeated stimulus presentations for increasing stimulus contrast are presented in Figure 1 (A-F, upper). In response to simple step current or very low contrast stimuli ($< \pm 0.17$ nA) very few spikes events occur at approximately the same time in each trial resulting in very low reliability measures. However, as contrast is increased, there is increasing banded structure in the raster plots corresponding to reliable spike train events. These events are binned according to spike time and the resulting histograms are shown in

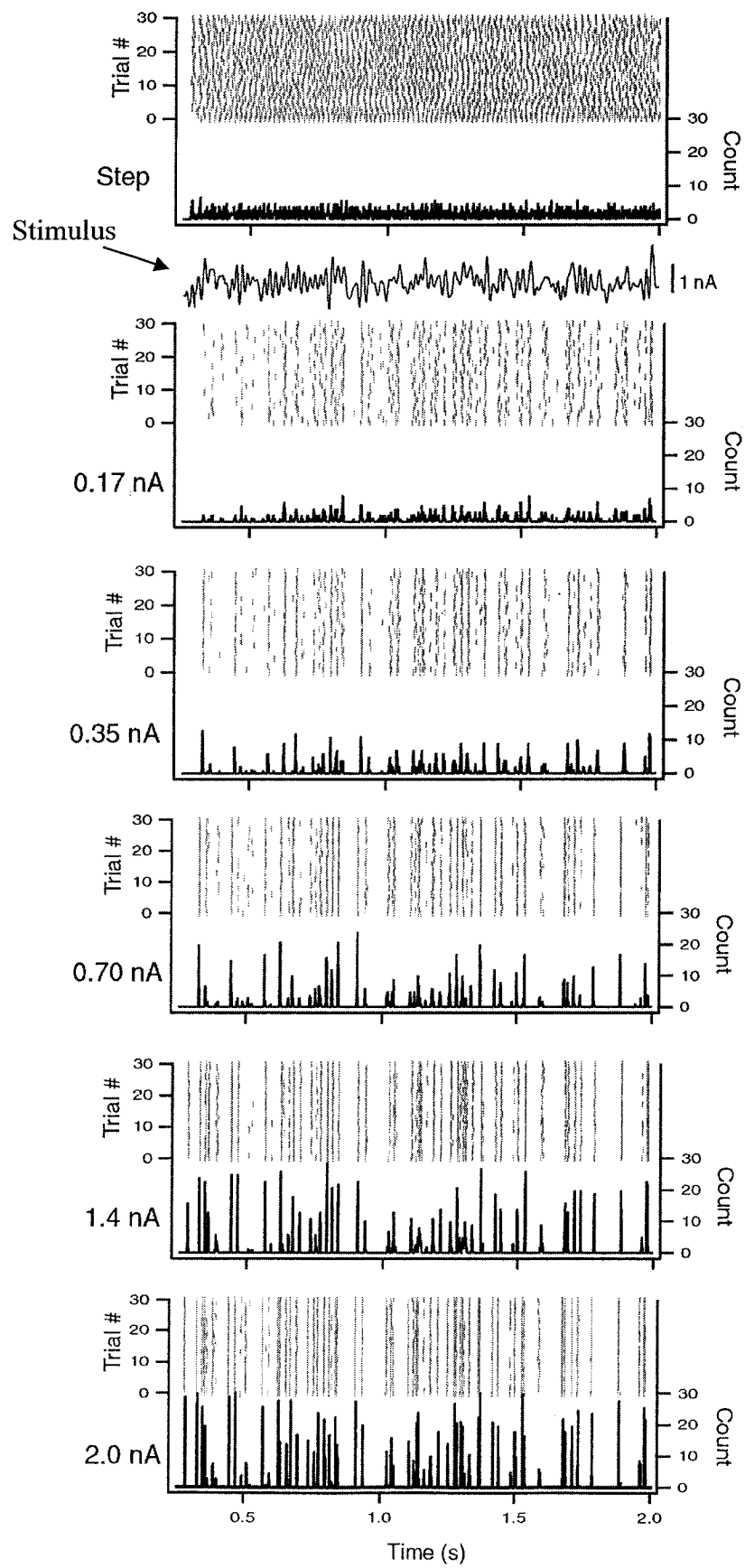


Figure 1: Neural responses to repeated presentations of a frozen noise stimulus.

These results are from a single neuron tested with repeated presentations of same noise stimulus. The cell was tested at the following stimulus contrasts (nA): 0, 0.17, 0.35, 0.70, 1.4, and 2.0 as indicated on the left hand side of each panel. The raster plots corresponding to the spike times are presented in the upper half of each panel. The stimulus is the trace between the panels corresponding to 0 and 0.17 nA. The time was binned in 0.5 ms windows. If a spike occurred on a given trial in a given bin, a count was added to that bin. The resulting event histograms are presented in the lower half of each panel (right axis). Events were defined as consecutive non-zero bins in these histograms. Note the increasing banded structure in the raster plots and number of reliable events with increasing contrast.

Figure 1 (A-F, lower). Threshold for reliability was set such that if a spike occurred in 66 % of trials, then that event (defined by consecutive non-zero bins) was considered reliable. The overall measure of reliability corresponds to the number of spikes occurring in reliable events divided by the total number of spikes. When single cells ($n=8$) were tested at multiple contrasts, the reliability increased with stimulus contrast (Figure 2A). The most significant increase in reliability corresponds to increasing contrast from 0.35 nA to 0.70 nA both in single cells ($P<0.01$, paired t-test) and across all cells and contrasts ($P<<0.01$, ANOVA) tested (asterisks, Figure 2B).

In response to stimuli of increasing contrast, the variability of the spike times from trial to trial decreases and thus the precision of the neural response increases. An example of this is shown for a single reliable event in Figure 3. As contrast increases, the histograms and the corresponding Gaussian curves fits become narrow. When standard deviation of the Gaussian decreases the precision increases such that low sub-millisecond values correspond to highly precise events. The average precision of all reliable events for each cell as well as the mean precision over all cells are presented versus contrast in Figure 4. The precision significantly increases between 0.35 and 0.70 nA when comparisons are made between contrasts in a single cell ($P<0.01$, paired t-test).

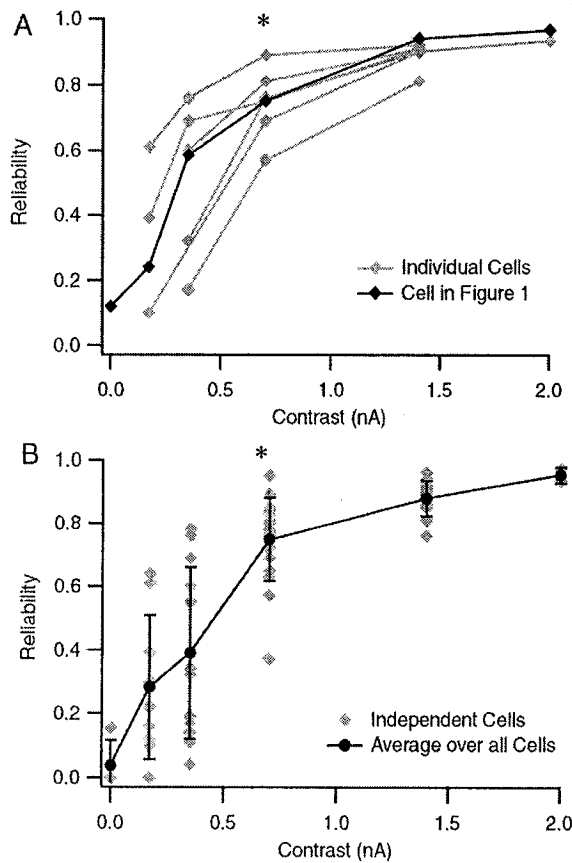


Figure 2: The reliability of spike timing versus stimulus contrast.

A) The reliability of individual cells tested with multiple stimulus contrasts (grey diamonds and lines). The reliability at each contrast for the cell presented in Figure 1 is shown in black. B) In addition to those cells in A, several cells were tested with only one or two contrasts. The reliability of these cells was consistent with those tested in A and are thus incorporated in the presentation of data from all cells tested (grey diamonds). The mean reliability of the full data set and the standard deviation is shown in black (circles and lines).

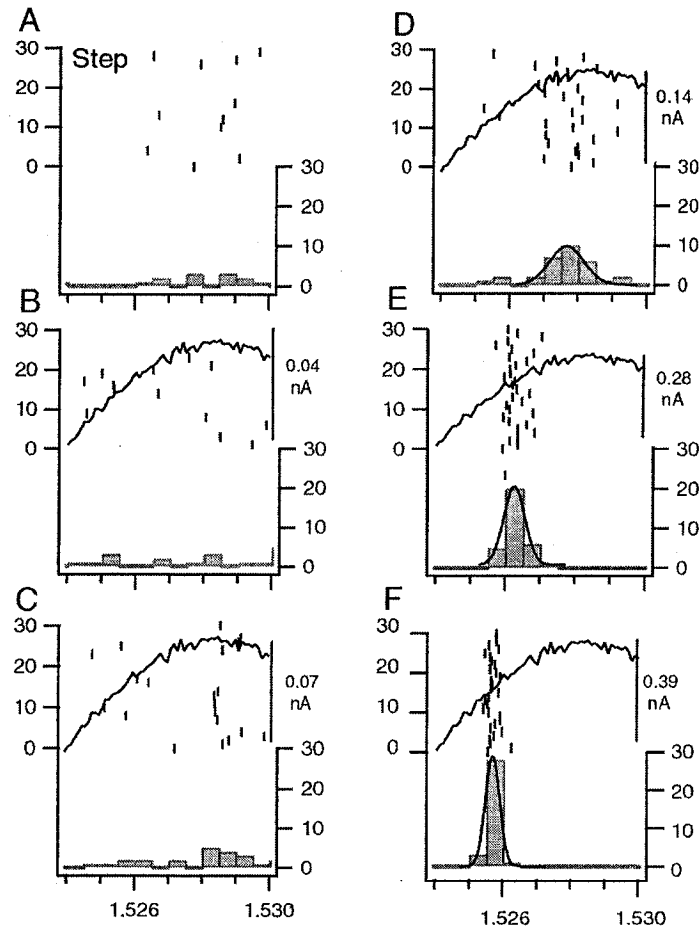


Figure 3: The measurement of precision at multiple contrasts in a single cell.

The measurement of the precision of a single event at multiple contrasts is presented in A-F. The cell is the same as that presented in Figure 1. The raster plots are presented in the upper half of each panel and the corresponding spike time histograms are presented in the lower half. The stimulus feature that elicits the spike is shown as a trace in the upper half of each. Note that the scale bar corresponds to the amplitude at the peak of the stimulus event. The stimulus contrasts (nA) tested are as follows A-0, B-0.17, C-0.35, D-0.70, E-1.4, F-2.0. The event is not considered reliable until the stimulus contrast reaches 0.70 nA so precision is not measured in A-C. The distribution of spike times (0.5 ms bins) for the event in D-F is fit with a Gaussian the width of which corresponds to the precision of the event. As contrast increases, the width of the Gaussian (ms) decreases, and spike timing is more precise.

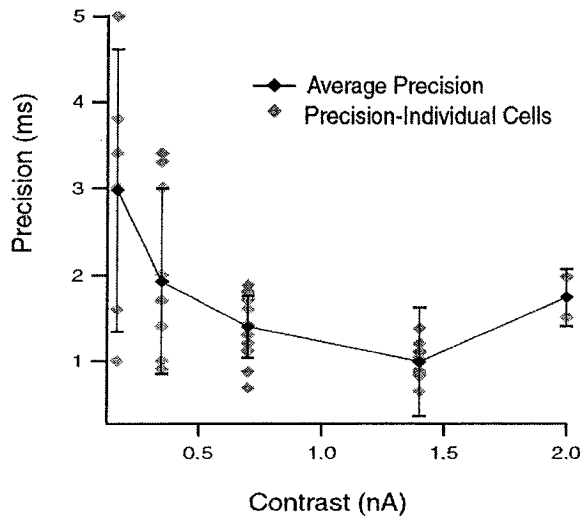


Figure 4: The precision of spike timing versus stimulus contrast. A)

The precision of all individual cells tested with multiple stimulus contrasts (grey diamonds). The mean reliability of the full data set and the standard deviation is shown in black (circles and lines). Lower values (ms) correspond to highly precise neural responses. Precision increases with stimulus contrast.

However, comparisons between the populations of cells tested at the two contrasts using ANOVA were not significant ($P=0.12$). This may in part be due to the fact that at low contrasts there are not enough reliable events to get an accurate measure of precision resulting in high degree of variability over the population of cells tested.

Analysis of Burst Events

Burst events were identified as two or more spikes occurring with an ISI less than 10 ms. The timing of burst events was defined by the time of the first spike of the burst. Stimulus contrasts less than 0.70 nA did not elicit sufficient burst fractions for analysis (see Table 1, Chapter 3). At higher contrasts the burst events were considered reliable (Table 1) but less reliable than isolated spike events (see P-Values, Table 1). However, the precision of burst events was both high and comparable to that of isolated spikes.

Contrast (nA)	Burst Events		Isolated Spikes		B versus IS	
	Reliability	Precision (ms)	Reliability	Precision (ms)	P-Value Reliability	P-Value Precision
0.70	0.68 ± 0.16	1.8 ± 1.4	0.77 ± 0.09	1.4 ± 0.3	<0.05	$>>0.05$
1.4	0.73 ± 0.19	0.8 ± 0.5	0.89 ± 0.06	1.0 ± 0.6	<0.01	$>>0.05$
2.0	0.81 ± 0.15	0.6 ± 0.02	0.95 ± 0.03	1.7 ± 0.3	n/a	n/a

Table 1: The reliability and precision of burst events for contrasts that elicited sufficient numbers of bursts for analysis. Comparisons were made between the reliability of isolated spikes (IS) and bursts (B) and the results of the paired t-test for each contrast are presented, P-values <0.05 indicated that the reliability of isolated spikes was significantly higher than that of burst events. P-values $>>0.05$ indicated that the precision of burst and isolated events was not significantly different. At the highest contrast there were an insufficient number of cells for this analysis.

The precise timing of spike train events was further investigated in a subset of the cells that were stimulated with a single 100 s trial of the noise stimulus. The average stimulus event that elicited an isolated spike or burst (obtained as described in Oswald et al., 2004) is presented in Figure 5A. The times of burst or isolated spike events with respect to the peak of the upstroke was assessed. The mean spike time for each event type corresponds to $t=0$ in Figure 5A. The distribution of these differences for each spike train event and stimulus event pair were compiled in the form of histograms such as the one presented in Figure 5B. Positive values indicate that the events were initiated on the upstroke while negative values indicate that the events occurred on the down stroke. In the cells tested, the majority of bursts (grey bars, $87 \pm 10\%$) are initiated on upstrokes while isolated spike events occur on both upstrokes and down strokes (black thick line).

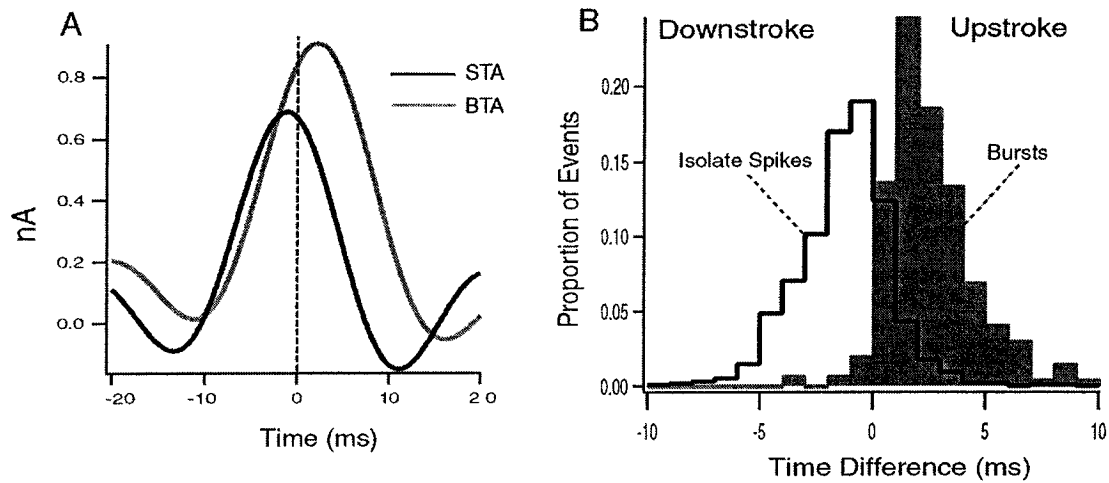


Figure 5: The timing of burst and isolated spike events with respect to the peak of the stimulus event. A) The burst (grey) and spike (black) triggered average (obtained as described in Oswald et al., 2004). The dashed line at 0 represents the average spike time with respect to the peak of the average stimulus event. B) The distributions of the differences between the time of the maximum of the stimulus event and time of the isolated spike (black, no fill) or time of the first spike of the burst (grey fill). Positive differences mean spike events occurred on the upstroke while negative differences correspond to down strokes. The Y axis is the proportion of isolated spike or bursts events that occur at each time difference. Results are for a representative cell stimulated with 0-60 Hz Gaussian noise for 100 s.

These results suggest that bursts are biased to initiate on upstrokes. Since bursts do not occur with low stimulus contrasts, bursts comprise a subset of spike train events that correspond to higher amplitude stimuli. When contrast increases, the amplitude and slope of individual stimulus events increase. As a result, the neuron reaches spike threshold faster, the latency to spike with respect to the peak of the stimulus upstroke (dashed vertical line) decreases and spikes occur earlier on the upstroke (Figure 6). The decreased latency to spike increases duration of time that upstrokes depolarize the neuron following the first spike and enables multi-spike events (bursts) to occur on a single upstroke (Figure 6 B, C). There is also a contrast dependent decrease in the time between the first two spikes of the burst (compare Figure 6 B, and C). These results suggest that

burst initiation is dependent both on the timing of the first burst spike and the amplitude of the stimulus.

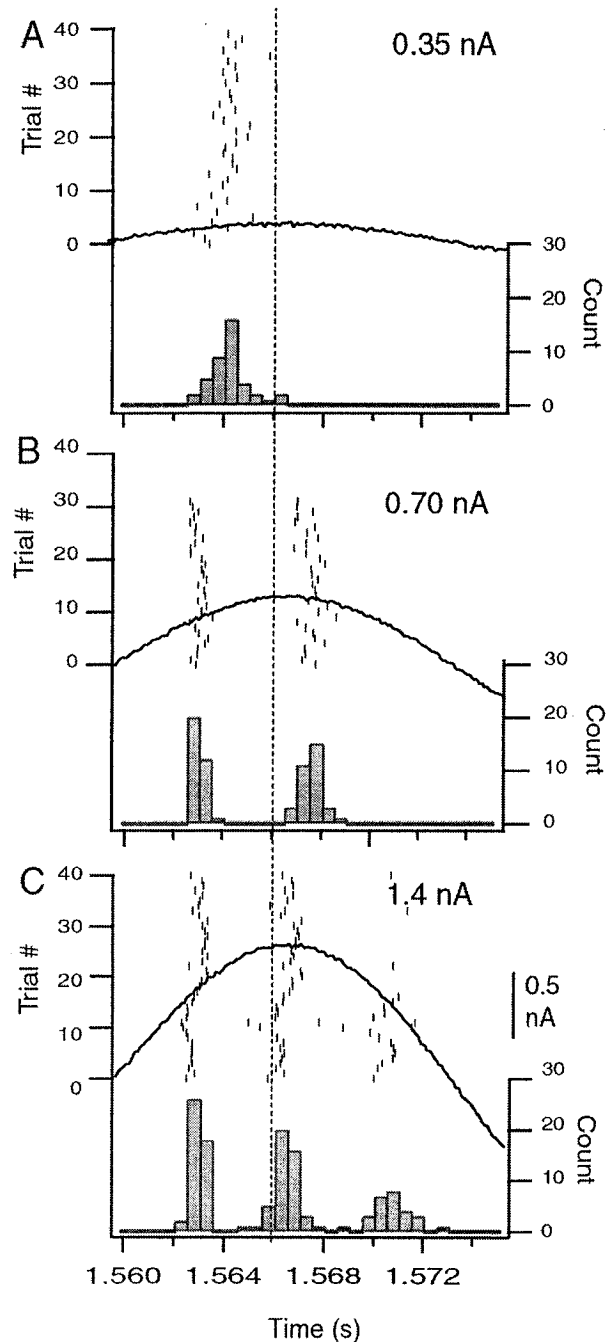


Figure 6: Spike latency with respect to the peak of the stimulus event decreases with contrast. The results of a single cell tested with increasing contrast are presented in A-C. The raster plots are presented in the upper half of each panel and the corresponding spike time histograms are presented in the lower half. The stimulus feature that elicits the spike is shown as a trace in the upper half of each. Note that the scale bar is 0.5 nA in all cases. The vertical dashed line corresponds to the time of the stimulus peak at all contrasts. Note the leftward shift in the timing of the first spike in each event as well as the recruitment of additional spikes (bursts) in B and C.

Discussion

The results of this study demonstrate that spike train events, both isolated spikes and bursts, are reliable and precise. Both reliability and precision increase with stimulus contrast. In the previous chapter, bursts were shown to code for low frequency stimulus features while isolated spikes coded for the full range of stimulus frequencies. When a stimulus of comparable frequency content and contrast to the stimulus used in the previous chapter was presented in the current study, both bursts and isolated spikes were highly reliable and precise events.

As stated previously, a number of studies have assessed the reliability and precision of spike train events. Spike timing has been shown to be related to the slope of the membrane potential at threshold (Azouz, 1999). This is particularly important with regard to the spike timing reliability and precision in response to dynamic stimuli such as those used in the present study. Two factors that will increase the slope of a dynamic stimulus are 1) increasing the stimulus contrast and 2) increasing the high frequency content of the stimulus (Mainen, 1995). In the present study increasing stimulus contrast increased spike timing reliability. An informal analysis of ELL pyramidal cell responses suggested that the neurons fire less reliably in response to low frequency noise stimulation ($f_c < 20$ Hz, data not shown). However, since the reliability of ELL pyramidal cell responses *in vivo* appears to be dependent both on the frequency content and spatial extent of the stimulus (see Figure 2, Chacron, 2003), the frequency dependence of reliability and precision may be more complex than *in vitro* studies indicate.

The reliability and precision of spike timing can also be influenced by interactions between time-scales in the stimulus and internal resonance time-scales of the neuron such

as the preferred firing rate or sub-threshold membrane oscillations (Fellous, 2001; Haas, 2002; Hunter, 2003). It has also been shown that phase locking in model cells (Masuda et al., submitted) and reproducible spike timing in ELL pyramidal cells (Doiron, 2003) can be induced when stimulus time scales are resonant with the time scales of external network phenomena such as feedback. Finally, the reliability and phase locking of spike events can be influenced by persistent sodium and sub-threshold potassium conductances (Svirskis, 2002; Schreiber, 2004). In ELL pyramidal cells, persistent sodium currents have been shown to affect burst threshold (Doiron, 2003) and to contribute to excitatory synaptic input (Berman, 2001). However, it is presently unknown how these currents might affect spike or burst reliability or precision.

In addition to assessing reliability and precision, this research also demonstrated the following burst characteristics: 1) bursts do not reliably occur at low contrasts suggesting bursts have a higher threshold for occurrence than isolated spikes, 2) bursts events are less reliable than isolated spikes, and 3) bursts are biased toward initiation on upstrokes.

The reduced reliability of bursts events is best explained with respect to the factors that determine the probability that a neuron emits a spike. A spike occurs when the membrane potential of the neuron surpasses the voltage threshold at which the autocatalytic opening of sodium channels results in an action potential event. The sub-threshold membrane potential is governed by external input and intrinsic neural activity. In order to fire a single spike per upstroke, the membrane must be sufficiently depolarized by the stimulus to cross spike threshold. To fire more than one spike per upstroke the stimulus has to be either extremely strong (see Figure 8, Oswald et al., 2004)

or of sufficient amplitude and duration to summate with the intrinsic depolarizing after potential (DAP) current to cross threshold. In the present study, multiple spikes occurring in response to a single upstroke are produced by the latter of these mechanisms. Since the stimulus is constant from trial to trial, the factors that determine the probability of producing one spike, whether an isolated spike or the first spike of the burst, for a stimulus event in a given trial, are comparable assuming the fluctuations in the membrane potential following long periods of quiescence are constant. However, the second spike of the burst is linked to the initial spike by the DAP. Thus, the probability of eliciting two or more spikes for a single stimulus event is subject to the timing of the first spike of the burst with respect to the stimulus, and the variability of the membrane potential following the initial spike, specifically the amount of after-hyperpolarization and the amplitude and time course of the DAP. Since a burst event is defined by the occurrence of two or more spikes, the reliability of a burst event is related to the probability of the second spike occurring. In the case of electrosensory pyramidal cells, the probability of eliciting a second burst spike for an optimal burst stimulus is high and so burst events are reliable. However, the probability of producing at least one spike for any stimulus event is higher, thus isolated spike events are more reliable than burst events.

This result, combined with previous studies, suggests an apparent paradox. If burst spikes are less reliable than isolated spikes, why are they better indicators of the occurrence of stimulus upstrokes (Gabbiani, 1996; Metzner, 1998; Oswald, 2004)? The answer is in the timing of the first spike of the burst as compared to that of an isolated spike with respect to the peak of the stimulus. The mechanism of eliciting a burst biases bursting toward initiation on the upstroke. The DAP amplitude peaks in the soma

approximately 3-7 ms after the first spike (Figure 7; Turner, 1994; Lemon, 2000). When the first spike occurs on the upstroke, the time course of the DAP enables the DAP current to summate with the stimulus current during the depolarizing and peak phase of the stimulus event (Figure 7, upper arrow), provided the stimulus event is of sufficient

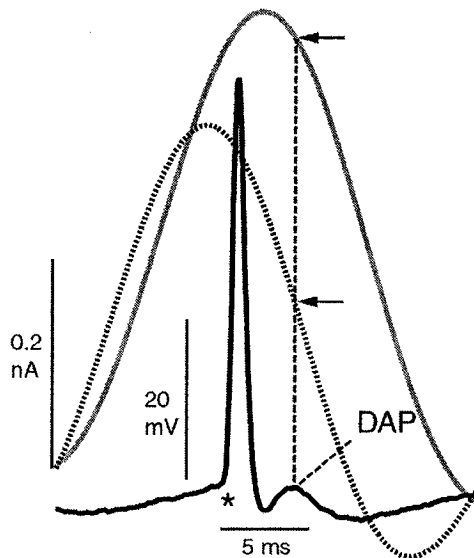


Figure 7: Schematic of the dependence of the recruitment of additional spikes on the timing of the first spike of the burst.

The spike (black dashed) and burst (grey) triggered averages presented in Figure 5 are redrawn here. A sample spike and DAP are also shown (black). The spike threshold (*) is aligned to the mean spike time with respect to the STA and BTA ($t=0$ in Figure 5). The time of the peak of the DAP is illustrated by the vertical line. The current from the DAP would be expected to summate with the BTA at a higher value of input current (upper arrow) than the STA (lower arrow) enhancing the probability that stimulus events similar to the BTA elicit multiple spike events

duration (i.e. low frequency stimuli). If the spike occurs at or after the stimulus peak, the DAP summates with the down stroke (Figure 7, lower arrow) of the stimulus when there is less stimulus current and a lower probability of eliciting a second spike. In the case of high frequency events, the duration of the stimulus is such that the DAP almost always coincides with the stimulus down stroke and, as a result, only a single spike is produced. Since bursts can only be initiated on upstrokes and isolated spikes can occur on both upstrokes and down strokes, bursts are better indicators of whether a stimulus upstroke occurred.

The results of this study demonstrate that spike timing is an important aspect of pyramidal cell coding. Not only do pyramidal cell spike trains reliably and with high fidelity transmit information about the frequency content of stimuli in the timing of isolated spike and burst events, but the timing of spikes with respect to the time course of the stimulus also influences whether an event is coded with a burst or an isolated spike. These results are consistent with the proposed idea that electrosensory afferents code time varying changes in stimulus intensity in their firing rate while electrosensory pyramidal cells code the occurrence of stimulus events in the timing spike train events (Metzner et al, 1998).

Chapter 3-A

Interval Coding I: Intensity Coding with Burst Inter-spike Intervals

Anne-Marie Oswald, Brent Doiron and Leonard Maler

First Manuscript of a two part series submitted
to the *Journal of Neurophysiology*

Division of Labor:

A.M. Oswald:	Data collection and analysis Conception of interval code Development and coding of interval code measure Interpretation of results, all text and figures
B. Doiron:	Development of interval code measure Interpretation of results

Abstract

High frequency inter-spike intervals such as those that occur during burst firing are hypothesized to be distinct features of the neural code. Although a number of correlations between the occurrence of burst events and aspects of the stimulus have been identified, the relationship between burst characteristics and information transfer is uncertain. In the present study we demonstrate that burst inter-spike intervals (ISI) are reliably and precisely correlated with the intensity of stimulus upstrokes. We found burst ISIs must differ by a minimum of 2 ms in order to discriminate differences in stimulus intensity. Finally, we demonstrate that coding with burst inter-spike intervals is restricted to short ISIs such as those generated in burst events and that the proposed interval code is distinct from rate and timing codes. Based on these results, we define and quantify a candidate interval code for the processing of sensory input.

Introduction

A number of strategies have been proposed for the coding of sensory input by neural spike discharge. The basic premise is that stimulus attributes are correlated with the spike train responses from single neurons or neuronal populations. Rate and temporal codes are two examples in which the firing rate or timing of action potentials is correlated with the intensity or event timing of the stimulus respectively.

In the course of sensory processing, spike trains often consist of isolated spikes, bursts of spikes, or both. It has been demonstrated that bursts are better indicators of stimulus events or features while isolated spikes provide an estimate of the stimulus (Gabbiani, 1996; Metzner, 1998; Sherman, 2001; Oswald, 2004). Specifically, bursts have been shown to be correlated with: low-frequency electrosensory stimuli (Oswald,

2004), the tuning frequency of auditory stimuli (Massaux, 2004), stimulus onset due to microsaccades (Martinez-Conde, 2002), regions of spatial opponency in visual receptive field maps (Reich, 2000) and specific features in natural visual scenes (Lesica, 2004). These results demonstrate that burst events can signal the occurrence of specific stimulus events.

These examples indicate that it is the timing of the burst event, usually the first burst spike, that is correlated with stimulus features. In some cases, including additional burst spikes beyond the first does not enhance spike train-stimulus coherence (Oswald, 2004) or stimulus estimation (Metzner, 1998). Yet bursts code more information per unitary event than isolated spikes (Reinagel, 1999) and spikes preceded by short, burst-like, inter-spike intervals (ISIs) convey more information than spikes preceded by longer ISIs (de Ruyter van Steveninck, 1988; Reich, 2000). Furthermore, the number of spikes in a burst can be correlated with the slope of the stimulus upstrokes (Keat, 2001; Kepecs, 2003) or the optimality of stimulus orientation (Debusk, 1997; Martinez-Conde, 2002). This potentially valuable information about the stimulus is lost if one only considers the timing of the first spike in a burst.

In the present study we show that the amplitude and slope of a dynamic stimulus are correlated with burst inter-spike intervals (ISIs) in electrosensory pyramidal cells. We expand on the feature extraction measure described by Metzner et al. (1998) and demonstrate that the upstroke characteristics coded by burst ISIs within a given range are highly discriminable from those of upstrokes associated with a different range of burst ISIs. This suggests that in addition to bursts detecting low-frequency stimulus events, burst ISIs also act as interval coders of stimulus intensity (Perkel, 1968). Finally we

provide a quantification of the additional information that utilization of a burst inter-spike interval code could provide about the stimulus.

Methods:

Transverse brain slices from the ELL of *A. leptorhynchus* were prepared in accordance with animal care protocols of the University of Ottawa as previously described (Berman, 1997). The slices were maintained at room temperature (20-22°C) in oxygenated ACSF for 1-2 hours prior to recording.

Intracellular recordings from 52 ELL pyramidal cells were made using 80-120 MΩ borosillate glass electrodes, pulled by a Brown-Flaming P-87 puller (Sutter Instrument, CA) and filled with 2 M KAc. All cells analyzed were identified as bursting cells using 2-4 sec constant current injections (0.5-1.5 nA) (Lemon, 2000). Recordings were made in the centromedial and centrolateral segments of the ELL. Although previous studies have reported differences in oscillatory activity between the segments (Turner, 1996), there were no segment-dependent differences observed for the measurements made in the present study (data not shown) and the results are therefore pooled. Upon impalement, the neurons were allowed to stabilize for ~5-10 minutes. If necessary, a constant 0.1-0.5 nA hyperpolarizing current was injected through the recording electrode to reduce spontaneous activity. The stimulus consisted of a baseline level of depolarization that resulted in a minimum firing rate of 10 Hz to which was added zero-mean, Gaussian noise with a cut off frequency of 60 Hz. The stimulus was a computer-controlled waveform (IgorPro and Pulse Control, Wavemetrics, OR) (Herrington, 1995) delivered via the recording electrode. The standard deviation of the injected noise (contrast) was varied from 0.35 nA to 2.0 nA. Pyramidal cell responses

were amplified (Axoclamp 2A, Axon Instruments, Burlingame CA), sampled at 10 KHz and stored for analysis (Igor Pro and Pulse Control).

Data Analysis: The neural responses were analyzed using Igor Pro and MATLAB (Mathworks, Natick, MA). At the onset of the stimulus, there was a transient non-stationarity in the cell responses that typically lasted a few seconds; this data was discarded.

Reliability and Precision of Burst ISIs: The reliability and precision of burst timing and burst ISIs was determined in a manner similar to those previously described (Mainen, 1995; Reinagel, 2002). A subset of cells (10) was stimulated with repeated presentations of shortened versions (3-10 s) of the Gaussian noise stimulus with a contrast 1.4 nA. Shorter stimuli were presented more often (30-50 trials) than longer stimuli (10-20 trials). The resulting spike trains were binned in 1 ms intervals, such that no more than one spike occurred per bin, and an event histogram (similar to the PSTH) was constructed. Due to the frequency content of the stimulus, there is typically only one spike or burst per upstroke. This resulted in 10-16 ms periods of quiescence that, in combination with the narrow bin-width facilitated the identification of single spike train events. An event was determined as consecutive non-zero bins in the event histogram. These consecutive bins were summed and if the resulting spike number was greater than 66% of the number of trials, the spikes within that event were considered reliable. The reliability of the cell was determined as the number of reliable spikes divided by the total number of spikes. These reliable events were then fit with Gaussians. The width of the Gaussians provided a measure of the precision of each event. The precision of the cell was the average precision over all reliable events. The reliability and precision of burst events was

determined based on the first spike of the burst. The precision of the ISIs was determined as the mean standard deviation of the ISIs for the reliable burst events.

Measurement of Interval Discriminability: The spike trains consisted of bursts and isolated spikes. Previous studies have partitioned the spike trains into burst and isolated spike events according to an ISI criterion determined from the valley of the bimodal ISI histograms (Debusk, 1997; Metzner, 1998; Bastian, 2001; Oswald, 2004). We used a similar criterion to identify burst events and then further partitioned these events according to their ISI value into groups based on the range value, τ , (ms). The first ISI event group (E_i , $i = 1$) consisted of all bursts with ISIs greater than or equal to the minimum ISI rounded down to the nearest integer value ($\mu_{\min}$, ms) and less than $\mu_{\min} + \tau$. Ensuing event groups ($i=2,3,\dots,n$) were determined according to ISI such that $(\mu_{\min} + (i-1)\tau) \leq \text{ISI} < (\mu_{\min} + i\tau)$. The duration of τ was systematically varied from 1-8 ms. The total number of features that could be coded by the burst events for a given τ is equivalent to the number of intervals $N = (\mu_{\max} - \mu_{\min})/\tau$, where $\mu_{\max}$ is the ISI criterion (ms) for determining burst events.

We then developed a variant of the feature extraction technique described in detail by Metzner et al. (1998) to obtain a measure of interval discriminability (I_D). Before the details of this method are described, we present an overview of the feature extraction technique.

The stimulus was time-binned at $\Delta t = 0.5$ ms to ensure that no more than one spike occurred per bin. For each bin $[t-\Delta t; t]$, we determined a stimulus vector, $s(t) = (s(t-100\Delta t), \dots, s(t))$, that corresponds to the stimulus waveform 50 ms prior to t . Typically, these vectors are grouped in distributions according to the occurrence of an event (E)-

$P(s | \lambda=E)$; where t equals the timing of the event. However, since we were interested in the stimulus that leads to a given ISI, we used the timing of the second spike of the burst to set t . We subdivided the burst stimulus vectors into ISI event distributions, $P(s | \lambda=E_i)$. The ISI boundaries of these distributions are determined by the range value, τ , chosen as described in the previous paragraph. The means (m_i) and covariances (Σ_i) of each distribution including the null distribution (no spike events, $P(s | \lambda=E_o)$) were estimated. The discriminability between each interval distribution- $P(s | \lambda=E_i)$ versus the null- $P(s | \lambda=E_o)$, and neighboring ISI distributions $P(s | \lambda=E_j)$ was determined where $j>0$ and $j \neq i$. The optimal feature vector (f) that separated these distributions was obtained by maximizing Fisher's linear discriminant function. Each stimulus vector was projected onto f and the separations between the projection distributions: $P(f^T \cdot s | \lambda=E_o)$, $P(f^T \cdot s | \lambda=E_i)$, and $P(f^T \cdot s | \lambda=E_j)$; were assessed using the linear classifier, $h_{f,\theta}(s) = f^T \cdot s - \theta$, where θ is the threshold for determining whether a stimulus feature (s) belongs to a given class (E_o, E_i, E_j). From this, the probability of correct detection, P_D , (classifying the stimulus as eliciting the correct response i.e. null response or an ISI within the given interval) and the probability of false alarm, P_{FA} , (classifying a stimulus vector as eliciting the incorrect response) were determined.

When P_D is plotted against P_{FA} the result is the receiver operating characteristic (ROC) curve (not shown), and from these values the error rate ε which is the minimum of $\frac{1}{2}P_{FA} + \frac{1}{2}(1-P_D)$ can be calculated. The error rate, ε , is a measure of how well the spike output of the pyramidal cell can convey information about the presence of stimulus features based the discriminability of spike train events. We use this measure as the basis

for our measure of discrimination (γ). Since ε is a value between 0-perfect prediction, and 0.5-chance, we compute;

$$\gamma = 1 - 2\varepsilon \quad (1)$$

such that γ takes a value between 0 (chance) and 1 (perfect).

The overall performance of an ISI group, E_i , depends on: 1) the discriminability of the group versus null (γ_{i0}); 2) the discriminability (γ_{ij}) of ISIs within E_i from those in neighboring groups E_j ; and 3) the proportion (p_i) of burst events that comprise E_i . Each ISI group has a discriminability value (D_i) between 0 and 1 that contributes proportionately to the overall summed discriminability of the burst ISIs (I_D):

$$D_i = \prod_{j \neq i}^N \gamma_{ij} \quad (2)$$

$$I_D = \sum_{i=1}^N D_i p_i \quad (3)$$

Equation 2 formally compares E_i to all other interval groups $E_{j>0}$ that occur at multiples of τ . However, for τ multiples greater than 1, we can approximate $\gamma_{i,i+2} = \gamma_{i,i-2} = \gamma_{i,i+3} = \gamma_{i,i-3} = \dots \approx 1$. This allows the approximation,

$$D_i \approx \gamma_{i,n} \gamma_{i,i+1} \gamma_{i,i-1} \quad (4)$$

Some ISIs occur infrequently either as a result of rare stimulus events or by chance so it is difficult to obtain a good estimate of the stimulus features corresponding to these intervals. Although these events may be highly discriminating, we cannot reliably measure them. For this reason we set a threshold on p_i such that, if these events are less than 1% of all burst events, they do not contribute to the I_D .

While the number of potential features that could be coded is equivalent to N , only the intervals with $p_i > 0.01$, can be considered as contributors to the code, N_C . Thus, the interval code, I_C , is quantified as:

$$I_C = N_C I_D \quad (5).$$

Results:

Burst Inter-Spike Interval (ISI) is Correlated with Stimulus Amplitude

ELL pyramidal cells were stimulated *in vitro* by intracellular current injection of a broadband (0-60 Hz) stimulus. Each cell was tested with one to four different stimulus contrasts which ranged from ± 0.35 nA to ± 2 nA. The resulting spike trains consisted of isolated spikes as well as bursts of spikes (Figure 1A). Increasing stimulus contrast resulted in an increase in the mean firing rate as well as the fraction of spikes that were contained in bursts (Table 1). The highest firing rates attained were still well below those produced *in vivo* by sensory stimulation (Bastian, 2002) suggesting that bursts are not due to non-physiological stimulus intensities and may therefore be relevant to *in vivo* sensory processing. At all contrasts, the majority of bursts were high-frequency (100-300 Hz) doublet events (Figure 1B) that corresponded to a prominent first peak in the bimodal inter-spike interval (ISI) histogram between 3 and 10 ms (Figure 1C). For this reason we focus on the ISIs of the doublets as opposed to the number of spikes in the burst as a candidate neural code. In Figure 1A, the doublet ISI appears to decrease with increasing stimulus amplitude. There is a corresponding leftward shift in the first peak of the ISI histogram (Figure 1C). These results suggest an inverse relationship between burst ISI and stimulus contrast that might be exploited as a part of the neural code.

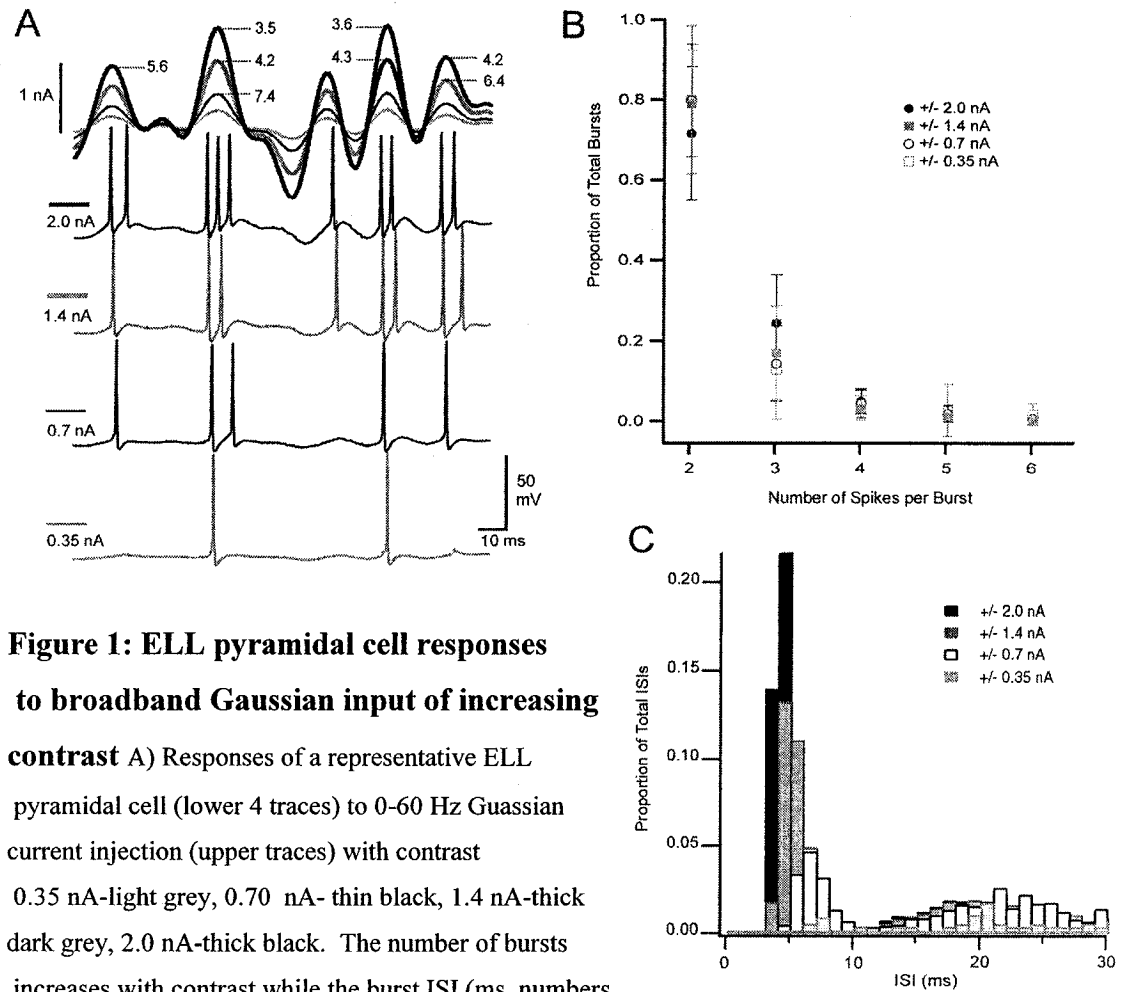


Figure 1: ELL pyramidal cell responses to broadband Gaussian input of increasing contrast

A) Responses of a representative ELL pyramidal cell (lower 4 traces) to 0-60 Hz Gaussian current injection (upper traces) with contrast 0.35 nA-light grey, 0.70 nA-thin black, 1.4 nA-thick dark grey, 2.0 nA-thick black. The number of bursts increases with contrast while the burst ISI (ms, numbers

in upper traces) decreases. B) The proportion of total bursts that are comprised of a specific number of spikes for all contrasts, 0.35 nA-open grey squares, 0.70 nA-open black circles, 1.4 nA-grey squares, 2.0 nA-black circles. Values are the average over the full data set, error bars are the standard deviation. Approximately 80% of all bursts are doublets C) ISI histogram for each contrast, 0.35 nA-light grey solid, 0.70 nA-open bars, 1.4 nA-dark grey solid, 2.0 nA-black solid. The ISI histogram has been truncated after the second mode in order to demonstrate the increase in the ISI width and shift left in the burst peak (<10 ms). The ISI histogram values extend to 120 ms.

To assess the stimulus parameters that may be encoded by burst ISIs we determined 40 ms stimulus regions centered on the timing of the first spike of each doublet. The intensity of the stimulus that gave rise to the burst ISI was determined as the average stimulus amplitude between the two doublet spikes. Burst ISI was plotted versus

intensity as shown in Figure 2 A for three of the contrasts tested in a single cell (triangles-0.70 nA, circles-1.4 nA, squares-2.0 nA). The individual stimulus events that elicited bursts were then grouped according to burst ISI by 1 ms increments from 3 ms to 10 ms to obtain a corresponding burst triggered average (BTA) for each ISI range (Figure 2 B). The amplitudes of the BTAs increased with decreasing burst ISI and are superimposed on Figure 2A; the symbols correspond to the stimulus contrast as above and the colors correspond to the BTAs of a given ISI in Figure 2B1-3.

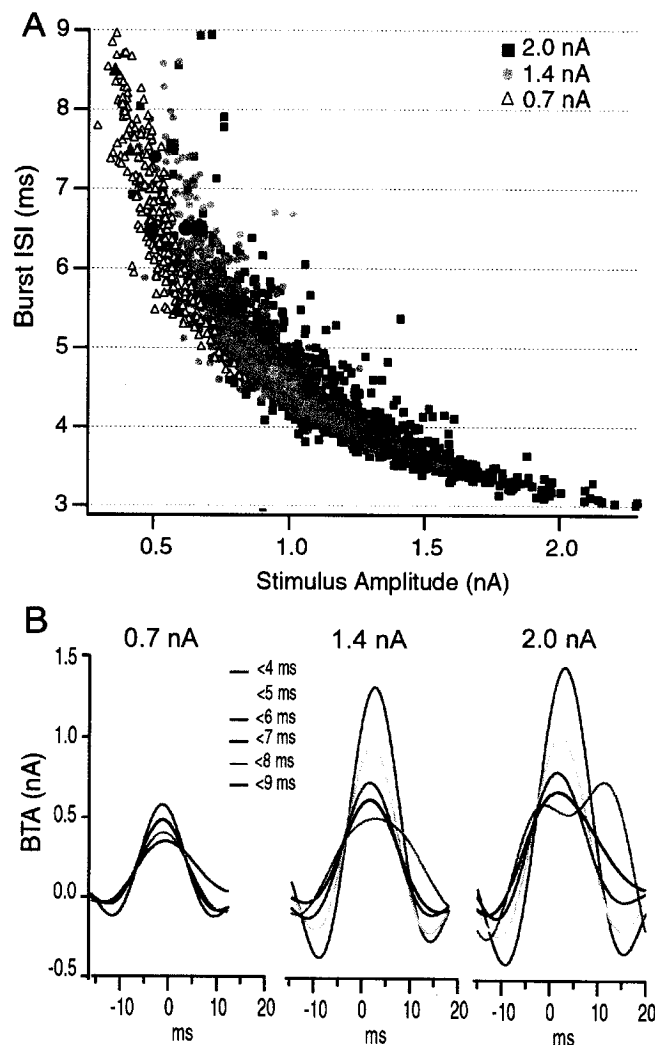


Figure 2: The correlation between the doublet ISI and stimulus characteristics.

A) Burst ISI plotted against the mean stimulus amplitude between the two spikes of the doublet for three contrasts, 0.7 nA-open triangles, 1.4 nA-grey solid circles, 2.0 nA-black solid squares, tested in a single cell. Note the increased range of ISIs and stimulus intensities with contrasts greater than 1.4 nA. B) The burst-triggered averages for each ISI group (1 ms windows) at each contrast tested for the cell in A.

In the majority of cells (37), these plots were linearly fit and the duration of the burst ISI was found to be negatively correlated with stimulus intensity (See R-values and Slopes, Table 1). The number of burst events and the range of ISI values significantly increases with higher contrasts (Table 1). For this reason, we will focus the remainder of the study on cells tested with σ greater than ± 1.4 nA.

Stimulus Contrast ($\pm$ nA)	0.35	0.70	1.4	2.0
n	17	29	25	10
Firing Rate (Hz)	11.6 ± 5.3	16.3 ± 6.3	21.9 ± 8.2	26.8 ± 8.6
BF	0.058 ± 0.074	0.181 ± 0.147	0.342 ± 0.187	0.453 ± 0.198
BEF	0.027 ± 0.034	0.080 ± 0.062	0.151 ± 0.081	0.189 ± 0.07
Minimum doublet ISI (ms)	n/a	4.6 ± 1.3	3.3 ± 0.89	2.9 ± 0.41
ISI versus Amplitude				
R-Value	n/a	-0.66 ± 0.15	-0.74 ± 0.096	-0.71 ± 0.11
Slope	n/a	-6.68 ± 2.24	-4.34 ± 1.26	-3.05 ± 0.64

Table 1: Pyramidal cell spike train statistics in response to 0-60 Hz Gaussian stimulation with varying contrast. The burst fraction (BF) is the number of spikes contained in bursts divided by the total number of spikes. The burst event fraction (BEF) is the number of burst occurrences divided by the total number of spike train events (isolated spikes plus burst events). Each burst counts as a single event regardless of the number of spikes it contains. The minimum doublet ISI is for burst events with ISIs < 10 ms. The R-value and the slope of the linear fit between burst ISI and stimulus event amplitude is reported for each contrast. The abbreviation n/a corresponds to occasions when there were insufficient bursts for analysis.

ELL bursts have been shown to be correlated with stimulus upstrokes both *in vivo* and *in vitro* (Gabbiani, 1996; Metzner, 1998; Oswald, 2004). Thus, we also investigated the relationship between burst ISI and the slope of the stimulus upstroke. The average slope between the two doublet spikes was weakly related to the ISI ($R: -0.39 \pm 0.19$,

slope: -11 ± 7.0 ms/nA) and varied between positive and negative values with many values near zero. This suggested that the burst spikes straddle the peak of a stimulus upstroke. This was verified by ascertaining the location of the maximum stimulus amplitude with respect to the time between two doublet spikes. The results are presented in the histogram in Figure 3A. This distribution has a peak between spike 1 (S1) and spike 2 (S2) showing that approximately 86% of doublets straddle stimulus peaks. There is also a peak at S2, demonstrating that ~12% of doublets occur entirely on the upstroke, but very few (2%) occur on the downstroke (S1). Based on these results, we found that the average slope between the first spike and the maximum of the upstroke is correlated with burst ISI ($R: -0.65 \pm 0.12$, slope: -31 ± 7.7 ms²/nA, $n=20$, Figure 3B). In addition, the average slope 5 ms preceding the first spike of the burst was correlated with burst ISI ($R: -0.61 \pm 0.08$, slope: -12.4 ± 1.9 ms²/nA, $n=20$).

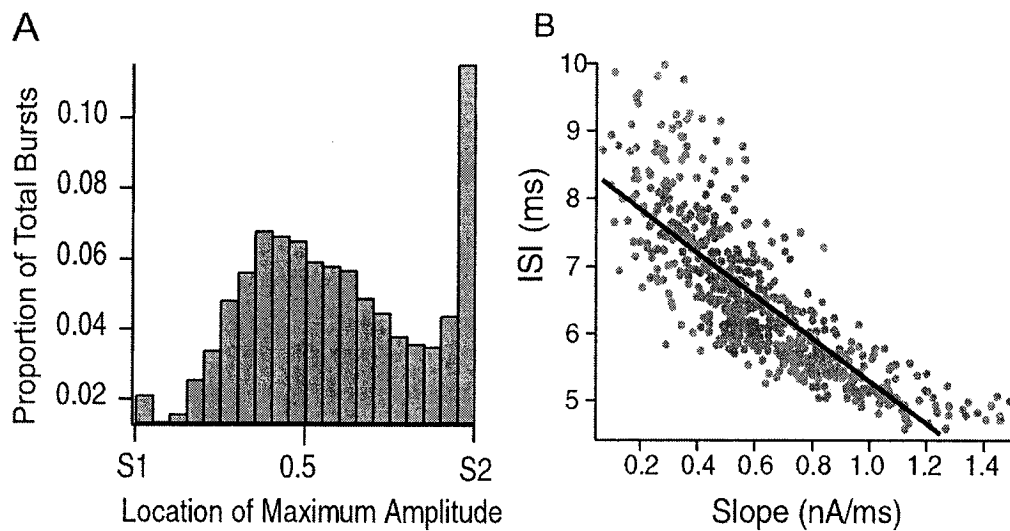


Figure 3: Relationship between burst ISI and the slope of stimulus upstrokes.

A) The location of the maximum stimulus amplitude between the two doublet spikes. Values on the x-axis correspond to the normalized distance between the time of the first spike ($S1=0$) and the time of the second spike ($S2=1$) of the doublet: $(tS2-tS1)/ISI$. The location of the stimulus maximum was determined by $(t_{max}-tS1)/ISI$, where t_{max} is the time of the maximum. Values on the y-axis are the proportion of maxima that occur at a given location between the two spikes for all doublets, from all cells analyzed (contrast 1.4 nA, $n=20$). These values were obtained by binning the locations and dividing each bin by the total number of doublets. Values near 0 on the x-axis correspond to bursts occurring at or near the stimulus peak and ending on downstrokes while values at 1 indicate that the burst occurs on the upstroke. Intermediary values correspond to doublets that straddle the stimulus peak. B) Relationship between burst ISI and the average slope between time of the stimulus maximum and the first doublet spike for a representative cell ($R: 0.815$, slope, $-31.8 \text{ ms}^2/\text{nA}$).

Burst ISIs are Both Reliable and Precise

These results demonstrate a strong relationship between burst ISI and stimulus parameters that is suggestive of an encoding of these parameters by the duration of the burst ISI. However, a requirement of any coding mechanism is that it is reliable. In a subset of cells, the reliability of burst ISIs was assessed for repeated presentations of the broadband stimulus with a contrast of 1.4 nA. The timing of burst events (as determined by the first spike of the burst) was both reliable (0.73 ± 0.19) and precise ($0.8 \pm 0.5 \text{ ms}$) (Figure 4A). The burst ISIs were very reliable from trial to trial (Figure 4A-C) as the mean standard deviation in ISI was $0.5 \pm 0.2 \text{ ms}$ over all trials. Finally, the relationship between ISI and stimulus amplitude or slope (not shown) seen in Figure 2 is demonstrable over repeated stimulus presentations (Figure 4 D). Thus bursts are a precise indicator of the time of occurrence of a stimulus feature (an upstroke) and the burst ISI is a reliable encoder of the upstroke intensity.

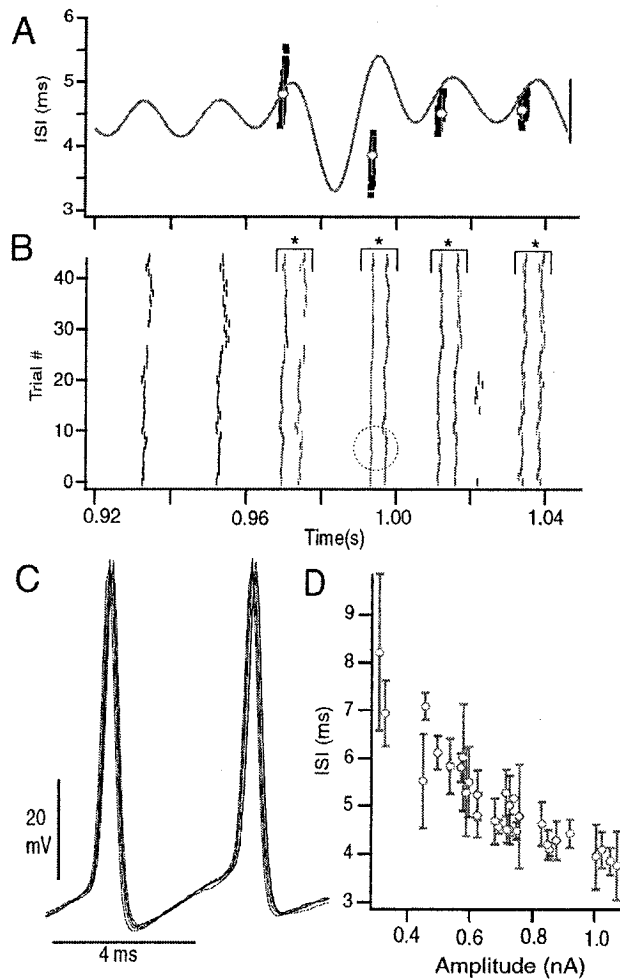


Figure 4: Reliability and precision of bursts and burst ISIs. Cells were stimulated with repeated presentations of frozen 0-60 Hz Gaussian noise (grey trace in A) with a standard deviation of 1.4 nA, scale bar: 1 nA. The results for a representative cell are presented in A-D. A) The ISI values for each trial are plotted (black squares) as well as mean and standard deviation over all trials—open circles and error bars. B) Raster plots in response to repeated presentations of stimulus in A. Burst events are indicated by brackets and asterisks. C) Neural responses (10) that gave rise to the spike times circled in B demonstrating the precision of the burst ISI. D) Mean ISI versus amplitude over all trials, error bars standard deviation of the ISI.

Evaluating the ISI Code

In addition to being reliable and precise, the information coded in a given burst ISI should be discriminable from that of other ISIs. The burst ISIs were partitioned into ISI event groups according to a range value (τ , ms) (see methods). Histograms of the corresponding amplitude and slope values for $\tau = 2$ ms groupings are presented in Figure 5. These results are for a single cell but comparable results were obtained for all cells (data not shown). The histograms suggest that amplitude and slope are discriminable

based on burst ISI. However, since these parameters are correlated in the stimulus ($R: 0.76 \pm 0.11$), it is questionable whether a single parameter is being coded or both.

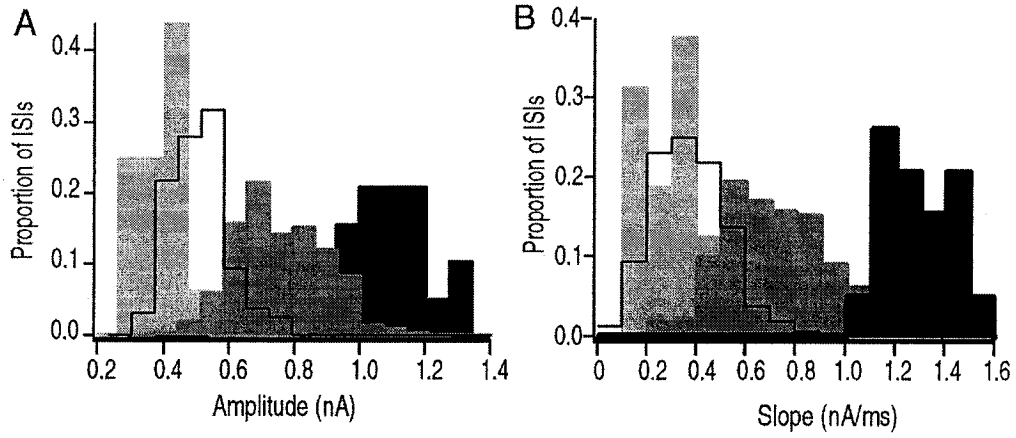


Figure 5: Distributions of amplitudes and slopes corresponding to burst ISIs

grouped according to 2 ms windows. Results are from the single cell presented in Figure 3. A)

Amplitudes corresponding to 3-5 ms ISIs- thick black, 5-7 ms ISIs - thick grey, 7-9 ms ISIs - thin black and 9-11 ms ISIs -thin grey. B) The average slope (as calculated in Figure 3) for each ISI group as defined in A.

We expand on the feature detection measure described by Metzner et al (1998) to demonstrate the potential of burst ISIs to discriminate and potentially code for stimulus intensity. This measure differs from simpler discrimination (d') measures in that the stimulus vectors discriminated are a segment of the dynamic stimulus over time rather than a single parameter such as amplitude or slope. These vectors incorporate both stimulus amplitude and slope. A detailed description of the interval coding measure (I_c) is outlined in the *Methods* section. Here, we present the feature discrimination results for burst ISIs that are partitioned into interval groups separated by multiples of 2 ms ($\tau = 2$ ms). We follow this with a demonstration of the effect of varying τ from 1 to 8 ms on the

overall discriminability of the neuron (I_D), the number of features coded (N_C) and finally our measure of interval coding (I_C).

The stimulus features (s) were partitioned into the following event distributions (E_i , $i=1$ to $(ISI_{\max}-ISI_{\min})/\tau$): 3-5 ms (E_1 , thick black), 5-7 ms (E_2 , thick grey), 7-9 ms (E_3 , thin black), 9-11 ms (E_4 , thin grey) based on the burst ISI and $\tau=2$ ms. The BTA for each event distribution is shown in Figure 6 A1. The first step in determining the discriminability of the ISIs of a given E_i is to ascertain how well those intervals detect the optimal feature for that E versus all non-spike (null) features. The optimal feature that maximizes the separation between each event distribution and the null distribution is presented in Figure 6 A2. Each stimulus vector was then projected onto the optimal feature and a prediction is made as to whether a burst with an ISI within a given distribution will occur or the null event will occur; this gives rise to the projection distributions in Figure 6 B. If the event distribution (solid lines) and the null distribution (dashed lines) overlap significantly, the ISIs of the event distribution are not considered discriminating. From these distributions the probability of false alarm and the probability of correct

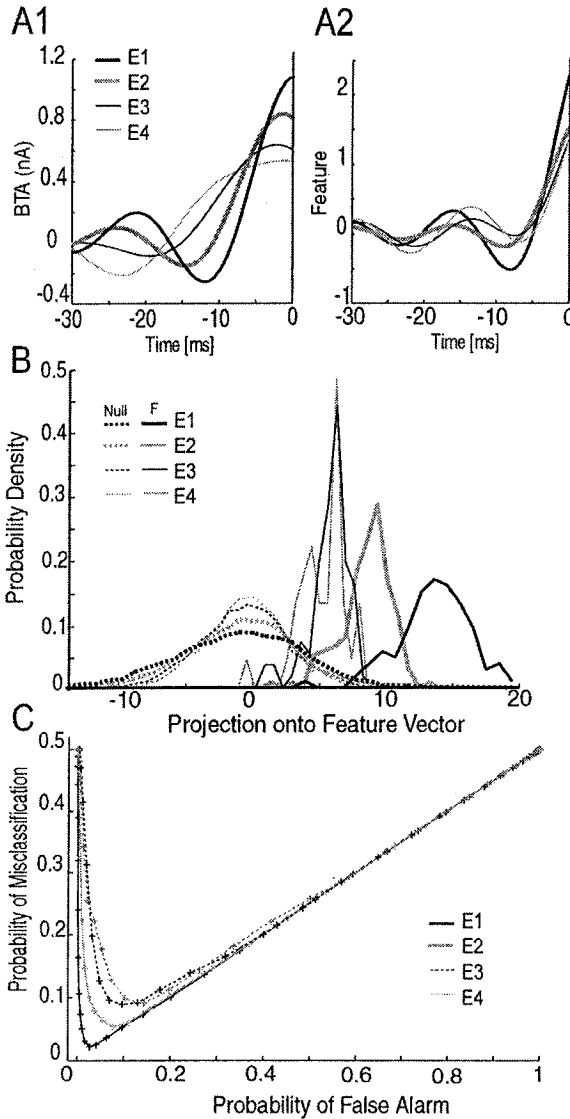


Figure 6: The discriminability of burst ISIs versus null.

The bursts were divided into event groups based on burst ISI and $\tau=2$ ms (see methods). The groups were as follows: E1: 3-5 ms ISIs (thick black), E2: 5-7 ms (thick grey), E3: 7-9 ms (thin black), E4: 9-11 ms (thin grey). A1) The burst-triggered average for each group. A2) The optimal feature for each distribution. In both A1 and A2, $t=0$ corresponds to the time of the second burst spike. B) The feature projections for each interval distribution (solid lines) versus the null distribution (dashed lines). C) The probability of misclassification versus the probability of false alarm for each event distribution. The minima of these curves are the error rates $\epsilon_{i,0}$.

detection can be determined. The overall probability of misclassifying a feature, P_ϵ , is given by $\frac{1}{2}P_{FA} + \frac{1}{2}(1-P_D)$ and plotted against the P_{FA} (Figure 6C). The minimum of this curve is the error rate, ϵ , a single value that corresponds to how well the spike output of the pyramidal cell can convey information about the stimulus upstroke based the discriminability of spike train events. The feature discrimination of each event distribution versus the null distribution is exceptional as indicated by the low ϵ values for each group: E1: 0.02, E2: 0.05, E3: 0.08 and E4: 0.09. We use this value as the basis for

our measure of discrimination, $\gamma = 1 - 2\epsilon$, such that γ values are numbers between 0 and 1, with 1 being perfect discrimination. The corresponding γ values were 0.96, 0.89, 0.82 and 0.82 respectively. These values were typical for all cells tested. Note that not only are the γ values high, there is gradation in the detection of shorter ISIs versus longer ISIs. This is not surprising given that bursts, especially those with very short ISIs, have a higher threshold for generation and are less likely to happen by chance.

The second step in calculating discriminability is to determine how separable the distribution of stimulus vectors that elicit a given ISI is versus the distributions of neighboring ISIs. The above procedure was repeated, but this time it was the distributions between neighboring intervals that were compared. Figure 7A shows the optimal features that separate the groups: $E_1:E_2$, black, $E_2:E_3$, grey and $E_3:E_4$, dashed. Figures 7 B1-3, are the corresponding feature projections (the line colours of the distributions are as described for E_1 - E_4 in the previous paragraph). The misclassification curve (P_ϵ versus P_{FA}) is presented in Figure 7C. The γ values between groups were as follows: $E_1:E_2$: 0.89; $E_2:E_3$: 0.82, and $E_3:E_4$: 0.74. Thus, not only do bursts within a specific ISI range detect specific stimulus features as compared to the null feature, but those stimulus features are well coded by a given ISI group as compared to neighboring groups.

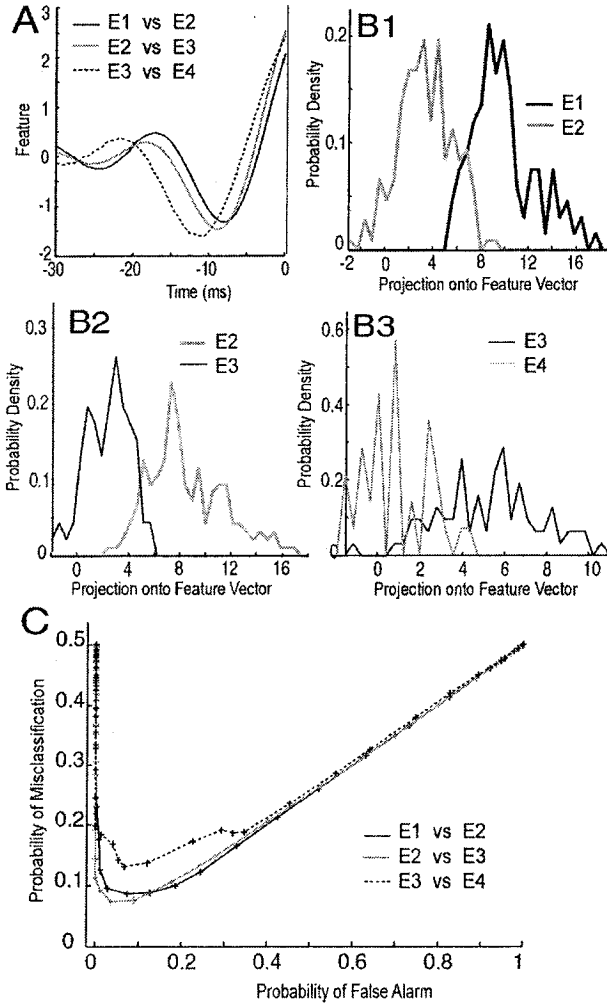


Figure 7: The discriminability between burst ISI distributions.

The bursts were divided into event groups based on burst ISI and a $\tau=2$ ms (see methods). The groups were as follows: E_1 : 3-5 ms ISIs E_2 : 5-7 ms, E_3 : 7-9 ms, E_4 : 9-11 ms. A) The optimal feature that separates two ISI distributions E_1/E_2 (black), E_2/E_3 (grey) and E_3/E_4 (dashed), $t=0$ corresponds to the time of the second burst spike. B) The feature projections for each interval distribution comparison 1: E_1 (black) versus E_2 (grey); 2: E_2 (grey) versus E_3 (black); 3: E_3 (black) versus E_4 (grey). C) The probability of misclassification versus the probability of false alarm for each event distribution. The minima of these curves are the error rates- ϵ_{ij} .

The measure of how well an ISI event group codes was determined as the discrimination of the E_i versus the null, γ_{in} , multiplied by discrimination of the E_i versus the nearest neighbor groups (E_j), γ_{ij} , weighted by the proportion (p_i) of bursts that comprise E_i (Equations 2 and 3). For the cell presented in Figures 6 and 7, the proportion of bursts in each ISI group was E_1 : 0.27, E_2 : 0.43, E_3 : 0.20 and E_4 : 0.10. Thus, the discrimination of group E_2 (5-7 ms) is equal to $\gamma_{2,n}\gamma_{2,3}\gamma_{2,1}p_2=0.27$ and group E_3 is $\gamma_{3,n}\gamma_{3,4}\gamma_{3,2}p_3=0.11$. Since there is not an ISI group smaller than 3-5 ms, nor greater than 9-11 ms the discrimination of these groups (E_1 , E_4) is given by: $\gamma_{1,n}\gamma_{1,2}p_1=0.21$ and $\gamma_{4,n}\gamma_{4,3}$

$p_4=0.05$ respectively. These values are then summed over all groups (Equation 3) to produce a measure of interval discriminability, I_D , for a given τ which has a maximum possible value of 1. The I_D in the present example was 0.63.

The mean I_D over all cells (solid circles) as well as the number of features that are coded (N_C , open circles) are plotted against τ (Figure 8A). Generally, I_D increases with increasing τ . Initially, I_D increases dramatically from 1 ms and 2 ms (**, $P < 0.01$, $n=20$). This is suggestive of 2 ms ISI differences being the minimal discriminability of the neuron. Further increases were also significant (**, $P < 0.01$; *, $P < 0.05$) between ensuing τ values.

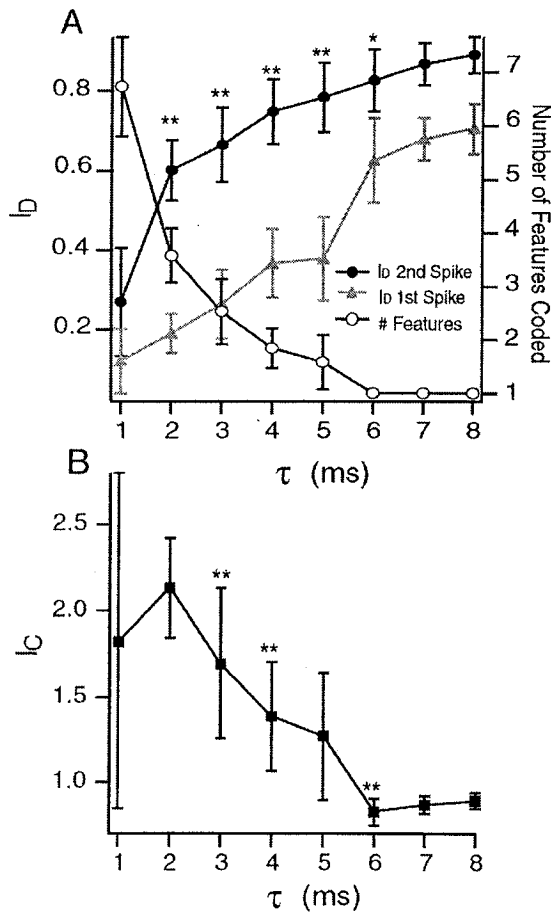


Figure 8: Interval Discrimination and Interval Coding versus τ A) The mean summed interval discriminability (I_D , left axis, equation 3) over all cells for each τ (ms) based on the second spike of the burst (black filled circles), the first spike of the burst (grey triangles). The mean number of features coded at each τ is also plotted (open circles, right axis) Error bars are the standard deviation and ** corresponds to a significant increase in discriminability (P-values < 0.01 , * : < 0.05) from the previous τ value. B) The mean interval coding (I_C) values for each τ ($I_D \cdot N_C$). The error bars are the standard deviation and ** corresponds to significant decrease in coding (P-values < 0.01) from the previous τ value.

As described in the methods section, the stimulus vectors that gave rise to bursts were determined as the region 50 ms preceding the second spike of the burst. Since previous studies have focused on the first spike of the burst as carrying the majority of information about the stimulus feature, we questioned whether a similar level of discriminability was possible when only the first spike is considered. In this case the stimulus vectors are the region 50 ms prior to the first spike but are still grouped according to ISI as previously described so that p_i and N_C remain the same. The $\gamma_{i,n}$ ranged from 0.65-0.8 indicating that the first spike of a burst is a reasonable discriminator of the occurrence of an upstroke. However, $\gamma_{i,j}$ values decreased substantially ranging from 0.05-0.4 indicating that the event distributions are no longer discriminable from each other. The resulting mean I_D values are shown in Figure 8 A, grey triangles. The most dramatic drops in the $\tau=1-5$ ms range, however, all values are significantly less (P-value $<<0.01$) than the I_D corresponding to the second spike of the burst. These results suggest that it is the properties of the region between the two spikes that is discriminated and thus coded by ISI.

The number of potential features, N_F , that could be represented by burst ISIs is equal to the number of event groups defined (see methods). The number of intervals consistently coded, N_C , corresponds to those groups that have a proportion of bursts greater than 1%. Unlike I_D , the N_C decreases with τ . Thus, $\tau=1$ ms yields the highest number of features (6-8) but also the lowest discriminability while $\tau > 6$ ms yield the highest I_D values but potentially code the lowest number of features (1-2). When I_D is multiplied by N_C , the resulting interval coding, I_C , values are scaled by N_C and no longer

constrained between 0 and 1. The I_C values for $\tau=2-5$ ms, which correspond to multiple features being discriminated, are significantly greater than when $\tau = 6-8$ ms and all bursts are treated as coding for a single feature (Figure 8 B). This implies that the amount of information about the stimulus extracted from the spike train could be significantly increased if a decoding neuron were able to discriminate differences of 2-5 ms between ISIs.

Limitations of Interval Coding

Since burst ISIs are easily identified as a peak in the 3-10 ms range of the bimodal ISI histogram, we focused on doublet ISIs as the units of the interval code. However, this does not necessarily preclude longer ISIs from interval coding. We investigated whether ISIs > 10 ms could also code additional information about the stimulus in their structure.

We focused on ISIs in the second peak of the ISI histogram (10-50 ms). These were grouped according to ISI into ISI event groups according to a τ of 10 ms. This longer τ allowed for sufficient counts in each distribution to do a discriminability analysis. The event triggered averages are shown in Figure 9A. It is apparent that the average intensity of the stimulus is equivalent for all event groups. The only distinguishing characteristics of these triggered averages is the timing of the peak that precedes the second spike at $t=0$. These peaks occur at the mean ISI value for each group. The ISI event distributions are discriminable from each other as indicated by low probability of misclassification values (Figure 9 B). However, it is clear from the optimal feature (Figure 9 B- inset) that it is the time interval between peaks that is being discriminated. Since the timing of each spike corresponds to the timing of a stimulus

peak, these intervals reflect the coding of the stimulus events by the timing of spike train events.

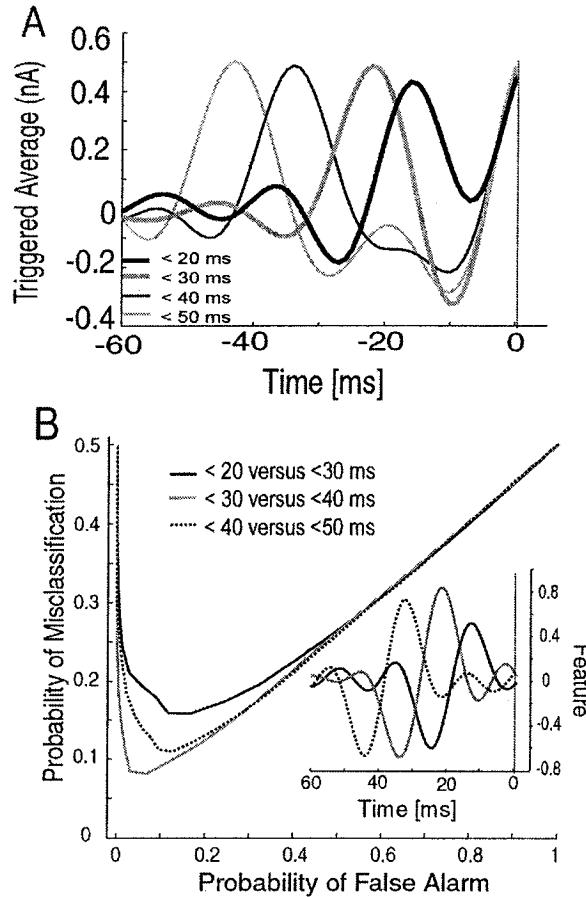


Figure 9: Discriminability of inter-spike intervals greater than 10 ms in response to 0-60 Hz stimulation. A) Triggered averages for ISIs <20 ms (thick black), <30 ms (thick grey), <40 ms (thin black), <50 ms (thin grey) where $t=0$ corresponds to the time of the second spike of the ISI. B) The probability of misclassification versus the probability of false alarm for between distribution comparisons: <20 versus <30ms (Black), <30 versus <40 ms (grey) and <40 versus <50 (dashed). The corresponding optimal features that separate these distributions are shown in the inset, $t=0$ corresponds to the time of the second spike of the interval.

These results suggest that the minimum requirement for an interval to code information about the stimulus in addition to event timing, is that the interval is shorter than the minimum period of the stimulus. We investigated whether this was the sole requirement by determining the discriminability of ISIs >10 ms in response to low frequency, 0-10 Hz Gaussian stimulation (Figure 10 A). Since the ISI histograms indicate that most intervals are less than 30 ms (Figure 10 B), we assessed event groups from 10-30 ms with a τ of 5 ms. The event-triggered averages are presented in

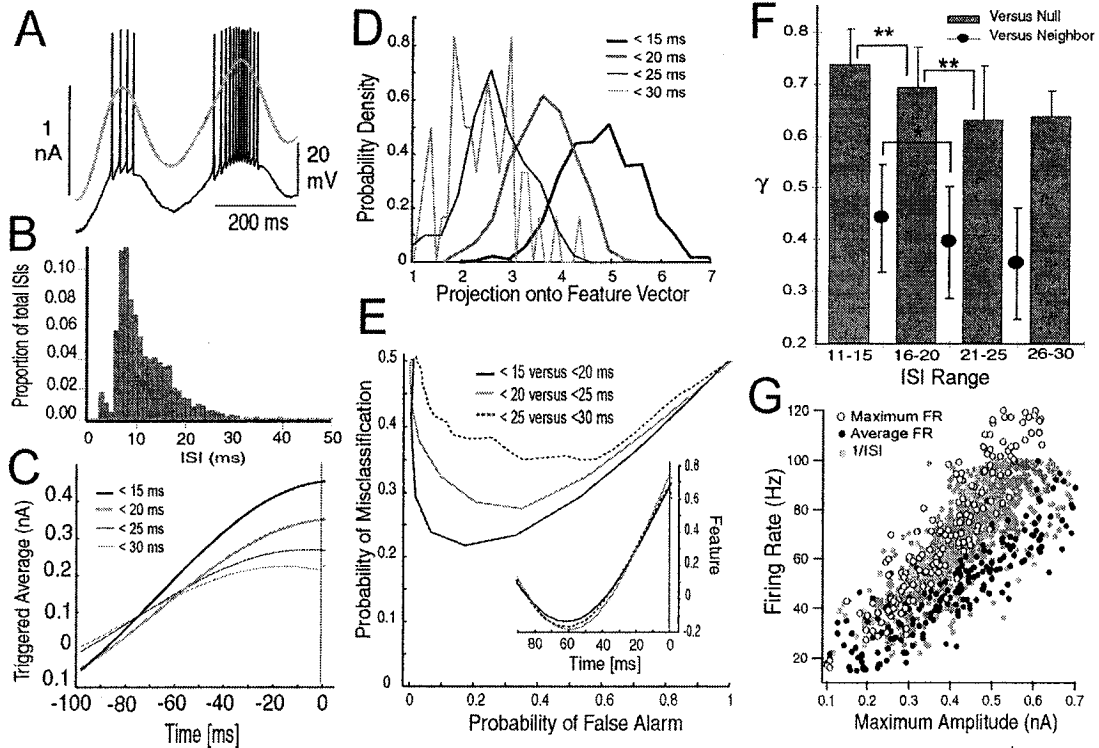


Figure 10: Discrimination by intervals greater than 10 ms in response to low frequency stimulation. A) Pyramidal cell response (black) to 0-10 Hz Gaussian stimulation (grey). B) ISI histograms of the pyramidal cell response shown in A. C) The interval-triggered averages for intervals (in ms) <15 (thick black), <20 (thick grey), <25 (thin black), and <30 (thin grey). D) The feature projections for comparisons between the interval distributions and the null distribution (not shown). E) The probability of misclassification versus probability of false alarm for comparisons between the distributions. The corresponding optimal features are shown in the inset. F) The mean discriminability values: $\gamma_{i,n}$ (grey bars) and $\gamma_{i,j}$ (solid circles) for all cells tested, error bars are the standard deviation. The discriminability of an interval versus null and versus neighboring intervals decreases significantly with interval length from 10 to 25 ms (**: $P < 0.01$, *: $P < 0.05$). G) The maximum instantaneous firing rate (1/minimum ISI per upstroke), open circles, and the average firing rate (black circles) versus the maximum amplitude of the upstroke. For comparison, the instantaneous FR of each interval (1/ISI) is plotted as well (grey circles).

Figure 10C. There appears to be a relationship between amplitude and interval, in that shorter ISIs do correspond to higher amplitude stimuli. However, projection of the stimulus vectors onto the optimal features (Figure 10 E-inset) that separate the event distributions show a high degree of overlap (Figure 10 D) that corresponds to a high

probability of misclassification (Figure 10E). The mean $\gamma_{i,n}$ (grey bars) and γ_{ij} (black circles) values for all cells tested are presented in Figure 10F. The discriminability of an interval versus null and versus neighboring intervals decreases significantly with interval length from 10 to 25 ms (**: $P < 0.01$, * $P < 0.05$). It has been shown that longer intervals required longer windows in order to be discriminable from neighboring intervals (de Ruyter van Steveninck, 1988). However, increasing τ to 10 ms only marginally improved discriminability (data not shown). One reason for this lack of discriminability is that many different ISIs occur on the same upstroke each corresponding to similar information about that stimulus event (Figure 10 A). Since the information for a given ISI is not discriminable from other ISIs it is unlikely that individual intervals provide more information about the intensity of the upstroke than the maximum instantaneous firing rate (1/minimum ISI of upstroke) or the average firing rate. These are plotted against the maximum amplitude of the upstroke (Figure 10 G- instantaneous FR: open circles, average FR: black circles). For comparison, the instantaneous firing rate of each interval (1/ISI) is plotted as well (grey circles). Thus, although these ISIs are shorter than the minimum period of the stimulus (100 ms), they comprise a rate code but not an interval code as defined in this study.

Discussion:

Inter-spike Intervals as a Candidate Neural Code

Studies of neural coding have yielded the following requirements for a potential neural code (for Reviews see: Perkel, 1968; Theunissen, 1995; Borst, 1999). First, a stimulus attribute such as frequency or intensity is correlated with a neural response such as the firing rate, spike timing, or the occurrence of bursts. Second, the neural response is

reliable and precise. Third, the neural response is decoded by a target neuron. As this third requirement is often difficult to ascertain, assumptions are made about the decoding of neural input. The correlation between stimulus attributes and the neural response is then quantified based on these assumptions to obtain measures of information transfer.

The concept of interval coding is not new. In a review of candidate neural codes, Perkel and Bullock (1968) outline a number of plausible means by which information may be transferred in the duration of the inter-spike intervals. In the present study, we demonstrate that burst ISIs are correlated with the slope and amplitude of stimulus upstrokes. We also show that bursts are reliable with millisecond precision, and that the variability in burst ISI is in the sub-millisecond range. The low variability in the ISI enables the discrimination of stimulus events based on burst ISI. Our results show that burst ISIs must differ by at least 2 ms in order for the stimulus features to be discriminated by ISI duration. It is possible that this lower bound on resolution may be due to under-sampling. Often the number of bursts is insufficient for a statistically significant number to occur at each ISI and contribute to our measure of neural discriminability. However, given the minimum precision of the spike train is ± 1 ms and the fact that it is difficult to conceive of the dynamics of a single synapse that could distinguish differences of 1 ms or less, this minimum seems appropriate.

We also identified two additional requirements of an interval code. It has been suggested that ISIs have to be shorter than the minimal time scale of the stimulus for a temporal *encoding* of stimulus features in the ISI (Theunissen, 1995). We verified this by assessing the discriminability of intervals that were greater than the minimum period of the stimulus. These intervals were unable to code additional information about the

stimulus in their ISI apart from the timing between two stimulus upstrokes. Thus, in the present case, longer intervals code the timing of stimulus events but do not encode any other characteristics. Effectively, in order to temporally encode the characteristics of stimulus events in the ISI there should be only one event coded per interval.

We investigated whether an encoding window shorter than the minimal period of the stimulus was the sole requirement for interval coding. In response to low frequency stimuli, the majority of ISIs are less than the minimum period of the stimulus and there appears to be a correlation between ISI and stimulus amplitude. However, because a number of different intervals occur on the same upstroke, they are not discriminable from each other. The information provided by these ISIs about the intensity of the stimulus event is the same as that provided by the firing rate. Thus, the number of intervals per event should also be limited to maximize discrimination.

The focus of this study was to identify and describe relevant stimulus features and determine how these features may be encoded. Although thorough information theoretic analysis of the interval code has yet to be performed (de Ruyter van Steveninck, 1988), our simple quantification of interval coding, I_C , demonstrates the potential for increased information transfer via burst ISIs, rather than provide a theoretically rigorous measure of information transmission. The quality of the interval code is dependent on the number of features coded and the discrimination of those features based on the neural response. The number of stimulus features is determined by the range of burst ISIs and the choice of the range value, τ . The discriminability of the intervals is dependent on the variability of the neural response which dictates a minimal τ . There is a trade off between the number of features coded and discriminability depending on the τ chosen. Shorter discrimination

windows yield a high number of features but lower discriminability while longer windows yield higher discrimination of fewer features. In the present study, this relationship is optimized by a $\tau=2-3$ ms where 4 and 3 stimulus features respectively are discriminable based on ISI. The resulting I_C values indicate that a significant amount of additional information could be transferred if a decoding cell could discriminate ISIs rather than treat all bursts as unitary events.

Burst Dynamics and Interval Coding

Short ISIs are more discriminable and require shorter windows of separation to be discriminable than longer ISIs (de Ruyter van Steveninck, 1988). In addition these ISIs carry more information per event than longer ISIs (de Ruyter van Steveninck, 1988). Bursts are ideal candidate coding elements because they often have different threshold requirements (for reviews see: Wang, 1995; Krahe, 2004) than isolated spikes and thus comprise a group of unique spike train events that may correspond to a subset of stimulus features. Furthermore, the autocatalytic aspect of spike generation within a burst produces short ISIs without an increase in the mean neural firing rate. In a previous study we demonstrated that replacing burst dynamics with high-frequency firing in order to produce comparable numbers of short ISIs reduces the discriminability of these events (Oswald, 2004). The ability to produce a range of burst ISIs that code for a range of stimulus intensities suggests a necessary interaction between the burst dynamics and the stimulus. This issue will be explored in the companion paper (Doiron, submitted).

Interval coding in the Electrosensory system

The electrosensory lateral line lobe (ELL) is the first processing center for the electric sense. ELL pyramidal cells receive electrosensory afferent input either directly

onto their basal dendrites (E-cells) or indirectly (I-cells) via inhibitory interneurons. Previous studies have stated that electrosensory afferent (P-unit) spike trains faithfully track amplitude modulations (AMs) of the electric organ discharge (EOD) (Wessel, 1996; Kreiman, 2000). By comparison, pyramidal cell spike trains are relatively poor linear estimators of electrosensory stimuli (Metzner, 1998) even under optimal stimulus conditions (Chacron, 2003). Nonetheless, ELL pyramidal cells are tuned to the frequency content, spatial extent and spatial correlation of the stimulus (Chacron, 2003; Doiron, 2003; Oswald, 2004). ELL pyramidal cells relay information from the periphery to higher brain centers. These results suggest that pyramidal cells pre-process input rather than relay an exact replica of electrosensory input.

ELL pyramidal cell bursts code the occurrence of low-frequency stimulus features (Oswald, 2004) such as prey or same-gender conspecifics. The intensity of these stimuli is related to the fraction of the pyramidal cell receptive field stimulated. Increasing the stimulus contrast is a simplistic mimic of the extent of receptive field stimulation. This stimulus does not account for distinctions between a smaller stimulus (prey) closer to the fish and a larger object further away, motion, center-surround or non-classical receptive field effects. Nonetheless, we have demonstrated that the intensity with which a pyramidal cell is stimulated can be encoded by burst ISIs. The short time scale of the burst ISI could offer an advantage for transmitting a significant amount of information quickly. Whereas a spike rate code might serve to estimate the stimulus amplitude of the entire time course of a low frequency event (upstrokes and downstroke, 100's of ms), information relating to the occurrence and peak magnitude of the upstroke is transmitted within the duration of the burst (less than 10 ms).

Decoding Burst ISIs

ELL pyramidal cell output is decoded in the torus semicircularis (TS), a midbrain structure (Carr, 1981). In a related electric fish, TS cells demonstrate frequency tuning both in response to stimulation applied at the skin as well as at the synaptic level. Interestingly, cells that respond to low frequency electrosensory input, facilitate high-frequency (burst-like) inputs from the ELL. Mid-range frequency inputs are suppressed through depression (Fortune, 1997, 2001, 2003). It has been proposed that interactions between time course and frequency thresholds for synaptic facilitation and depression could result in the selective transmission of inputs that have frequencies that are resonant with the synaptic dynamics (Izhikevich, 2003). Alternatively, certain burst ISIs may match a resonance timescale in the sub-threshold membrane dynamics of the post-synaptic neuron, which could enhance the transfer of specific ISIs over other ISIs (Izhikevich, 2003). ELL pyramidal afferents also synapse with toral interneurons that then synapse with toral principle cells (Maler, unpublished observations). The combination of plasticity and disynaptic interactions may further frequency filter ELL inputs as has been suggested for thalamocortical connections (Beierlein, 2002). Though it is conceivable that plasticity at ELL-torus synapses promotes the transfer of low frequency information by bursts, it is unknown whether plasticity, disynaptic interactions or subthreshold membrane dynamics occur with timescales that would enable the discrimination of different burst ISIs. Nonetheless, our results, as well as those from the visual system (de Ruyter van Steveninck, 1988; Reich, 2000) suggest that additional information about a stimulus can be contained in short inter-spike intervals, provided an appropriate decoder can be identified.

Chapter 3-B

Interval Coding II: Dendritic Modulation of the Interval Code

Excerpt from the study:

Interval Coding II: Dendrite Dependent Coding

Brent Doiron, Anne-Marie Oswald and Leonard Maler

This manuscript is the second in a two part series
submitted to the *Journal of Neurophysiology*

Division of Labor:

A.M. Oswald:	All simulations Analysis of data Interpretation of results, all text and figures
B. Doiron:	Development of Models Interpretation of results

Introduction

The results of Chapters 1-3 suggest that bursts code for the occurrence of low frequency stimulus events and that the burst inter-spike interval (ISI) codes the intensity of these stimuli. In this section, excerpts that comprise my contribution to a companion modeling study to Chapter 3A are presented. The results of this section demonstrate that burst coding may be modulated by changes in the dendrite-induced, depolarizing after potential (DAP). These results are of significant interest because the dendritic region responsible for the active backpropagation of dendritic spikes receives substantial and highly plastic excitatory and inhibitory feedback from higher brain centers as will be discussed in Chapter 4.

When an ELL pyramidal cell somatic spike actively backpropagates along the apical dendrite, the resulting dendritic spike is broader than the somatic spike enabling the additional current to be reflected at the soma as a depolarizing after potential (DAP). The Ghostbuster equations (Doiron, 2002) used in Chapter 1 model the “ping-pong” interaction (Wang, 1999) between dendrite and soma that results in burst responses. However, the proposed mechanism for the generation of multi-spike events via the interactions between the DAP and 0-60 Hz Gaussian stimulus (see Figure 7, Chapter 2) suggests that the full complexity of the burst mechanism is not utilized in response to these stimuli. In this section, a simpler model of spike generation and the ensuing DAP is presented. The effects of modulating the DAP amplitude on interval coding are then investigated.

Methods

Pyramidal cells were stimulated via intracellular current injection with 0-10 Hz or 0-60 Hz Gaussian stimuli as described in previous chapters. Pyramidal cell responses were modeled using the Ghostbuster equations as described in the thesis introduction and in Doiron et al., 2002. These simulations were similar to those of Chapter 1 with the following exceptions: persistent sodium currents were not modeled, simulations were conducted in the XPP simulation environment and the model parameters were as described in Doiron et al., 2002. The equations and results for simple LIF-DAP model will be described in the results section.

Results

The influence of the slow burst variable p is dependent on stimulus frequency.

As outlined in the introduction of this thesis, the ELL pyramidal cell burst mechanism involves three main components: 1) a dendrite-dependent DAP at the soma, 2) a potentiation of the DAP over the course of a burst event, that is hypothesized to be due to a potassium-dependent process and 3) a longer and dynamic refractory period in the dendrite than the soma that results in dendritic failure. In response to steady state depolarizing input, the burst evolves as a series of spikes with decreasing ISIs as a result of enhanced excitability due to the potentiating DAP. The burst terminates with a high frequency doublet and a large burst after-hyperpolarization (bAHP) (see Figure 7, Introduction). The evolution of the burst is dependent on the degree to which the dendritic spike broadens and the refractory period of the dendrite (Lemon, 2000; Noonan, 2003). Spike broadening is hypothesized to be due to a cumulative inactivation of the

dendritic potassium conductance which is represented by the slow variable, p , in pyramidal cell models (Doiron, 2001, 2002).

The burst responses to Gaussian stimuli (0-60 Hz) presented in the preceding chapter generally consisted of two spikes (see Figure 1, Chapter 3A) without the characteristic large AHP at burst termination. However, the cumulative inactivation that results in dendritic spike broadening generally requires many spikes to be activated. The effects of stimulus frequency on burst structure and the recruitment of cumulative inactivation as indicated by the slow variable, p_d , are shown in Figure 1 using experimental results and the Ghostbuster model (Doiron, 2002). The responses of an ELL pyramidal cell to low frequency (0-10 Hz) and 0-60 Hz Gaussian stimulation are presented in Figure 1A and B respectively. The burst events elicited by low-frequency stimuli are comparable to those seen in response to steady-state depolarization and are easily identified by large AHPs (*, Figure 1A). However, as stated, the majority of burst events in response 0-60 Hz stimulation are doublets lacking large AHPs (*, Figure 1B). The responses of the Ghostbuster model to comparable stimuli are presented in Figure 1 C and D. The corresponding values of the slow variable, p_d , which dictates dendritic spike broadening, are also plotted (thick black line). The slow variable becomes sufficiently inactivated in response to low-frequency stimulation producing a long multi-spike burst ($p_d < 0.1$, see Doiron et al., 2002). However, during 0-60 Hz stimulation the p_d values never approach those required for long bursts because the frequent stimulus down strokes hyperpolarize the cell and repetitively reset p_d . The histograms of the minimum p_d values following each model spike in response to 0-10 (grey bars) or 0-60 Hz (black line) stimuli are presented in Figure 1 E. High-frequency, burst-terminating doublets are

elicited at p values to the left of the arrow, which corresponds to $p_1=0.10$ (Doiron, 2002).

The p_d values often cross into this region in response to low frequency stimulation but very rarely in response to 0-60 Hz inputs.

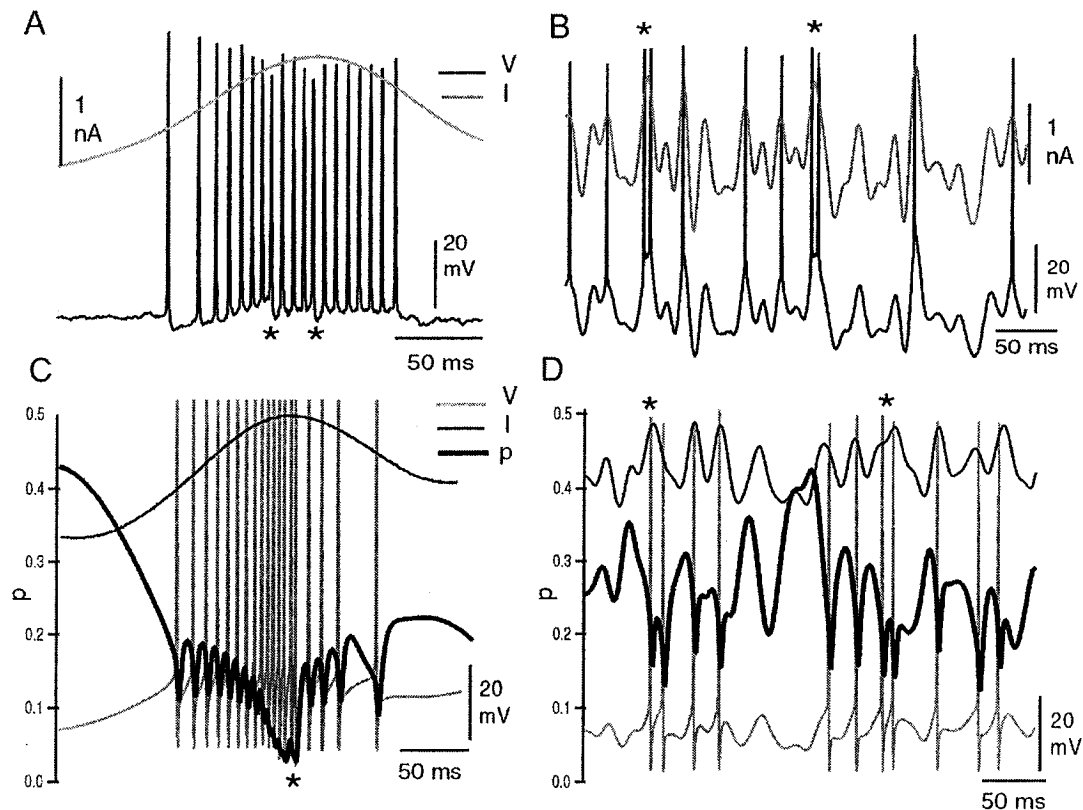
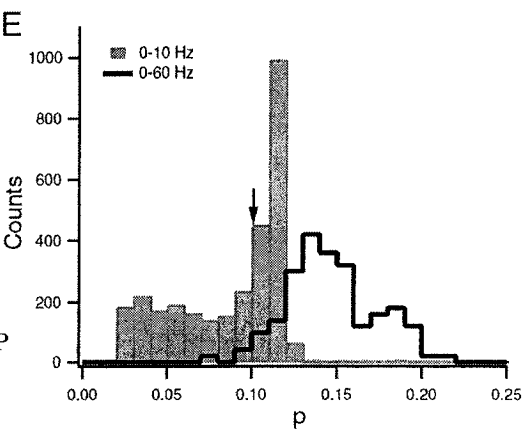


Figure 1: Analysis of the slow variable, p_d , in response to Gaussian stimulation.

A) ELL pyramidal cell response (V-black) to 0-10 Hz Gaussian input (I-grey). Burst AHPs are indicated by the *. B) ELL pyramidal cell response (V-black) to 0-60 Hz Gaussian input (I-grey). Doublets are indicated by the *. C) The response (V-grey) of the Ghostbuster model to 0-10 Hz input (I-Black). The burst AHP is indicated by the *. D) The response (V-grey) of the Ghostbuster model to 0-60 Hz input (I-Black). Doublets are indicated by the *. In both C and D the corresponding values of the slow variable (p) are plotted (thick black line).

Lower values correspond to increased inactivation of the dendritic delayed rectifier potassium channel and an increased probability of generating high frequency ISIs. At near zero values a high frequency doublet is elicited. E) Histogram of the minimum p values following each model spike for 0-10 Hz (grey) and 0-60 Hz stimulation (black). The arrow indicates the p value beyond which the high frequency doublets that result in dendritic failure are elicited (as indicated in Doiron et al, 2002). During 0-60 Hz stimulation the slow variable rarely reaches this value



Thus, the slow variable does not play a significant role in burst generation with 0-60 Hz stimulation. We propose that the majority of bursts in response to 0-60 Hz inputs are truncated by the rapid high frequency down strokes in the stimulus that reset the slow dynamics in the dendrite before long multi-spike burst events can evolve.

The dendrite has a dynamic refractory period which, like spike broadening, is dependent on multi-spike bursts (Noonan, 2003) and leads to dendritic failure. Although dendritic failures were reported in Chapter 1, they only occur in doublets with ISIs less than 5 ms. The analysis of the frequency dependence of the dynamic refractory period with respect to Gaussian stimuli is presented in the companion study to Chapter 3A (Doiron et al, submitted). This study suggests that the dynamic refractory period of the dendrite does not play a significant role in the doublet generation in response to 0-60 Hz inputs.

Finally, the Ghostbuster simulations do not produce a range of burst ISI values comparable to those seen in experimental data. The Ghostbuster ISIs ranged from 3-6 ms in response to 0-60 Hz stimulation (Chapter 1). This ISI range is too narrow to recreate the ISI coding values of experimental data (Chapter 3A). This may in part be due to the fact the two compartment model inadequately describes the spatial aspects of the dendritic compartment that contribute to the time course of the DAP.

The Leaky-Integrate and Fire + DAP Model

The analysis of the Ghostbuster model in response to 0-60 Hz input suggest a simpler approximation of ELL burst dynamics in response to stimuli that contain high frequencies. This model depends only on the generation of a DAP and does not require a

slow dendritic variable (potassium channel inactivation). Motivated by previous leaky integrate-and-fire (LIF) modeling of ghostbursting (Laing, 2003; Laing, 2003), we present the following description of ELL burst events in response to Gaussian stimuli:

$$\frac{dV}{dt} = -V + \gamma x(t - \tau_d) + I(t) \quad (1)$$

$$\frac{dx}{dt} = y \quad (2)$$

$$\frac{dy}{dt} = -\alpha^2 x - 2\alpha y + \alpha^2 \sum_i \delta(t - t_i) \quad (3)$$

Since Equation 1 is a standard LIF description of spiking dynamics, the model does not actually spike, rather the membrane potential (V) depolarizes until threshold ($V(t)=1$) and is then reset to zero. The spike time t_i is defined by $V(t)=1$ and the absolute refractory period is determined by τ_r . Every time $V(t)$ spikes, the DAP which is modeled by an alpha function excites the membrane after a time delay τ_d . The alpha function $x(t) = \alpha^2 t e^{-\alpha t}$ is the solution of the subsystem (x, y) with the initial conditions $x(0)=0$ and $y(0)=\alpha^2$ (Equations 2 and 3). The alpha function better represents the time course of the DAP that results from the spatial aspect of the dendrite than the Ghostbuster equations. The intensity of the alpha function (DAP) is scaled by γ . Analysis of model results was conducted with techniques described in Chapters 1 and 3.

The LIF-DAP model recreates pyramidal cell responses

A schematic of the model and a sample of the model responses to steady state depolarization and noise stimulation are shown in Figure 2. The contribution of the DAP to the membrane potential following a spike can be visualized by comparing the LIF-DAP model to the LIF without a DAP (Figure 2B). In the case of the LIF with the DAP the membrane potential rises to threshold faster than without a DAP. The LIF-DAP

model does not burst in response to steady state depolarizations (Figure 2C) because the slow dynamics that give rise to the full burst event are not incorporated in the model. Nonetheless, this model accurately recreates the neural responses to Gaussian stimuli presented in Chapter 1 and 3 of this thesis (Figure 2D, Figure 3). When the model is stimulated with 0-60 Hz Gaussian noise, the resulting ISI histogram is bimodal with a peak <10 ms corresponding to doublet ISIs that range from 3-10 ms (Figure 3A). In addition the doublets are coherent with low frequency stimulus events while isolated spikes are coherent with broadband to high frequency stimuli (Figure 3B). Finally, the doublet ISIs generated by the model are correlated with stimulus intensity and thus, the model has discriminability and interval coding values comparable to experimental results (Figure 3C). It should be noted that the initial condition of the model were set such that the output of the model matched the firing rate, burst fraction and ISI distribution of experimental data only. The model was not fit for coding measures such as coherence, discriminability or feature extraction and the evaluation of model performance with respect to these measures serve as a test of model performance.

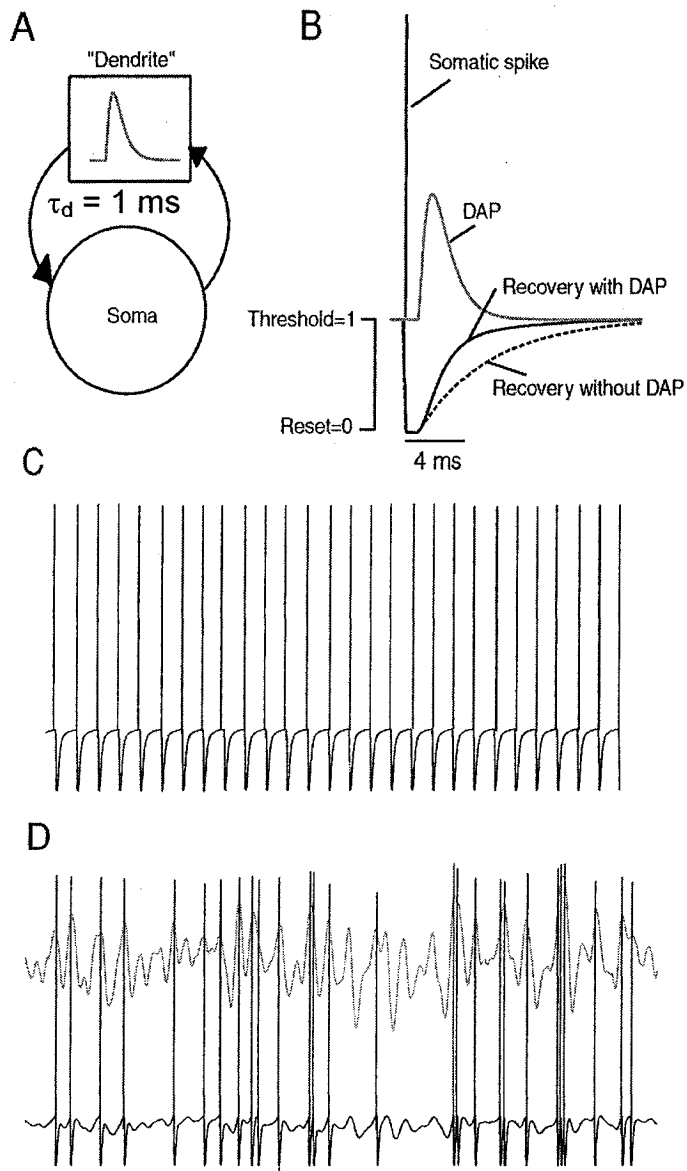


Figure 2: The LIF-DAP

model. A) Schematic of the LIF-DAP model. B) When the soma crosses threshold ($V(t)=1$), the somatic voltage is reset to zero and then passively rises exponentially back to rest (dashed line). An alpha function (DAP-grey) that represents the dendritic influence at the soma feeds back to the soma (as shown schematically in A) within 1 ms of the 'spike' and hastens the recovery to rest (solid line). LIF models do not model spike dynamics. The vertical lines representing spikes in this figure are added for illustrative purposes. C) The response of the LIF-DAP model to steady state depolarization is tonic firing, not bursting. D) In response to 0-60 Hz Gaussian input the model responds with comparable spike and doublet frequency as in experimental results.

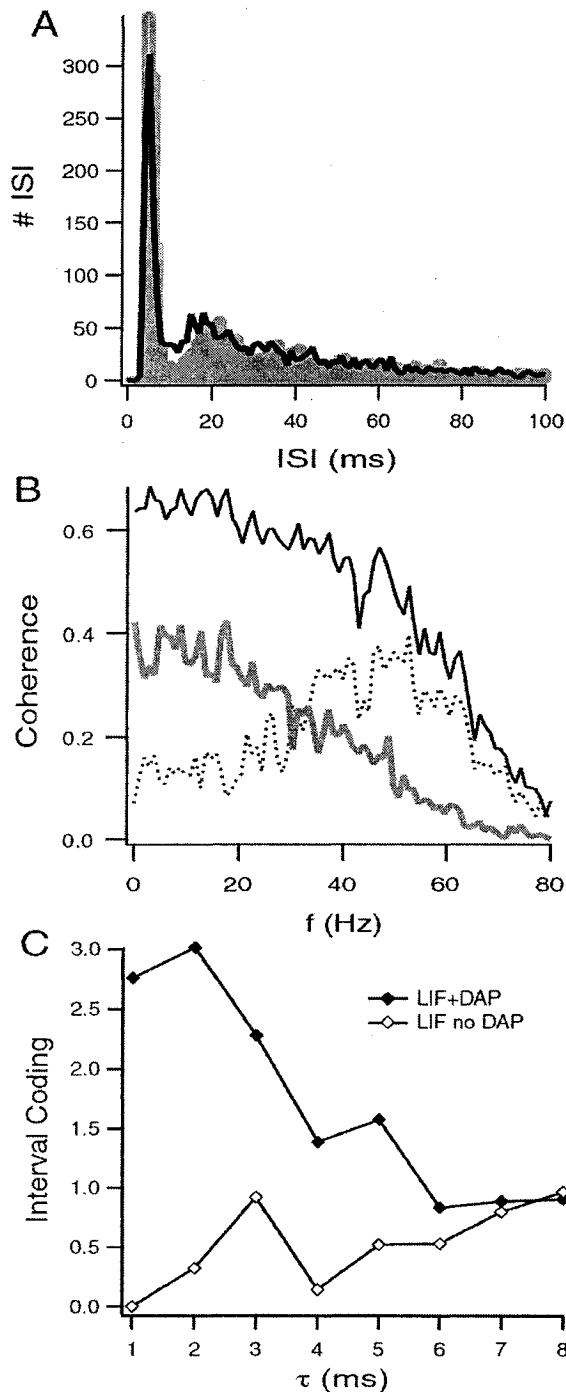


Figure 3: The LIF-DAP model has comparable coding characteristics to experimental results in response to broadband Gaussian inputs.

A) Bimodal inter-spike interval (ISI) histogram, model (black line) data (grey bars). B) The model recreates the parallel processing by isolated spikes (dashed line) and bursts (grey line) as well as the all-spike coherence of pyramidal cells *in vitro* (compare with Figure 1, Chapter 1). C) The model has comparable interval coding values for burst ISIs (black diamonds) (see Figure 7, Chapter 3A). The removal of the DAP current in the model suggests a dependence of interval coding on the dendritic feedback to the soma (open diamonds).

The Effects of Changing DAP Amplitude on Interval Coding

In the Ghostbuster model (as in true neurons) DAP amplitude is controlled by several dendritic voltage gated ion channels. One benefit of using this reduced model of bursting is that DAP amplitude can be scaled by changing a single scaling parameter (γ).

This study simply asks, “If the DAP amplitude is changed, without reference to the specific ionic mechanism involved, what is the effect on interval coding?” As γ is varied from 0 to 5.5 with 2.5 being the optimal value that recreates experimental results, the relationship between ISI and stimulus amplitude changes (Figure 4A). The range of ISIs that code the range of stimulus intensities is greatest at mid range DAP intensities (ISIs: 4-9 ms, $\gamma = 2.0-3.0$) but is decreased at γ values higher (ISIs: 4-7 ms) or lower (ISIs: 6-9 ms). The slope of the relationship between ISI and stimulus intensity also changes suggestive of a change in gain in the model response with changes in DAP amplitude. The effects of changing DAP amplitude on interval coding with ISIs are presented in Figure 4B. There is a range of DAP amplitudes for which adequate interval coding is possible ($\gamma = 1.5-3$). Outside this range, coding drops dramatically. These results suggest that factors that affect DAP amplitude change the gain of the neural response which in turn affects coding with burst ISIs.

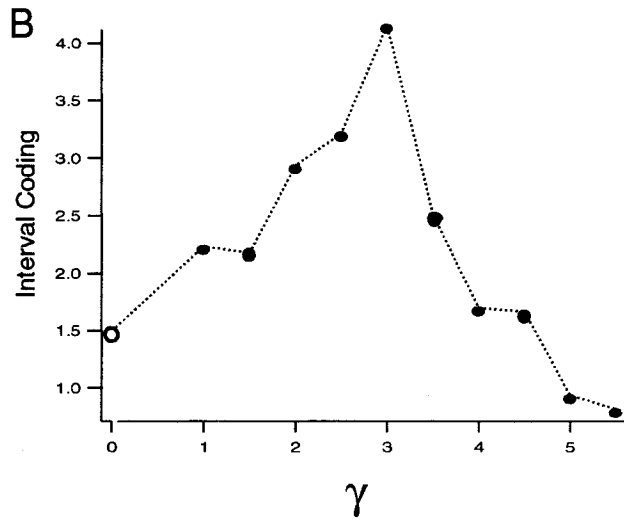
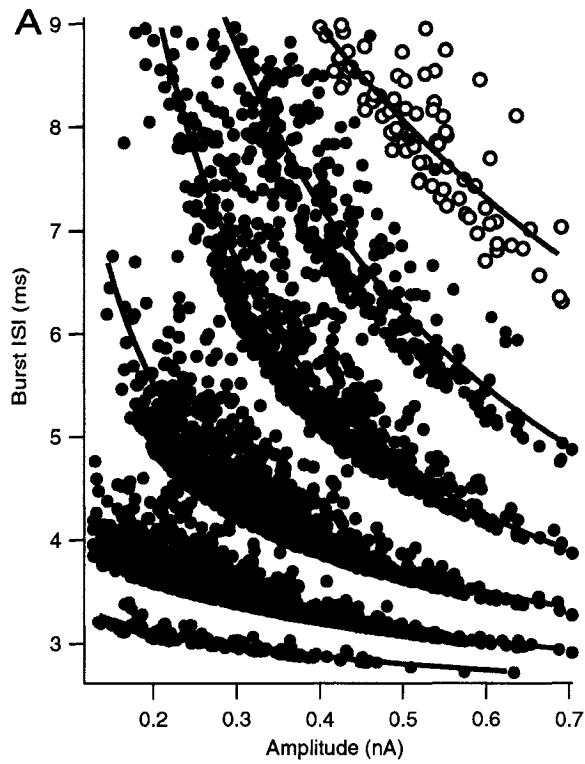


Figure 4: Scaling the DAP

amplitude alters interval coding. A)

The relationship between burst ISI and stimulus amplitude (as measured in Chapter 3) for DAPs scaled at different values of γ : 0- open circles, 1.5-blue, 2.5-black (control), 3.5-green, 4.5-eggplant and 5.5-cyan. B) Interval coding values versus the scaling factor γ . Coloured dots correspond to the DAP intensities shown in A.

Discussion

The reduction of the dendritic influence on the soma to a simple alpha function, allowed us to demonstrate that changes in DAP amplitude can affect interval coding by ELL pyramidal cells. The amplitude and time course of after-depolarizations at the soma are dependent on the magnitude and time course of the backpropagating dendritic spike. Recent studies have shown that the timing of dendritic synaptic input with respect to the backpropagating action potential, can influence the impact of the dendritic spike both at distal synapses and in the soma (Hausser, 2000, Stuart, 2001). The apical dendritic region responsible for the active backpropagation of somatic action potentials in ELL pyramidal cells receives a significant amount of excitatory and inhibitory feedback input from the nucleus praeminentialis (Pd) via the stratum fibrosum (StF) (Maler, 1981; Bastian, 1996a, 1996b, 1998a). Stimulation of the excitatory fibers of the StF has been shown to elicit bursts in ELL pyramidal cells (Turner, 2002). Recent work has shown that application of GABA_A agonists to the apical dendrite reduces the amplitude of the DAP reducing the somatic firing rate in response to current injection (Mehaffy, submitted). Thus, it is conceivable that synaptic inputs could enhance or inhibit backpropagation and affect DAP amplitude and that these effects could be affected by the synaptic plasticity described in Chapter 4.

Finally, since the reduced model reproduces the results of Chapter 1 and 3A, many aspects of pyramidal cell coding can be attributed solely to the influence of the DAP rather than the complete manifestation of the complex burst mechanism of ELL pyramidal cells. Depolarizing after-potentials have been reported in a number of pyramidal cell types that exhibit regular spiking and/or burst dynamics (Connors, 1990).

Thus, the DAP-dependent coding schemes demonstrated in electrosensory processing may be employed by a variety of cells and sensory systems.

Chapter 4

Dynamically Interacting Processes Underlie Synaptic Plasticity in a Feedback Pathway

Anne-Marie M. Oswald, John E. Lewis, Leonard Maler

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Division of Labor:

A.M. Oswald	<i>In vitro</i> data collection and analysis Development of the model Interpretation of results All text and figures (exception listed below)
J.E. Lewis	Model development and coding Simulations Interpretation of results Parts of model methodology text

Abstract

Descending feedback is a common feature of sensory systems. Characterizing synaptic plasticity in feedback inputs is essential for delineating the role of feedback in sensory processing. In this study, we demonstrate that multiple interacting processes underlie the dynamics of synaptic potentiation in one such sensory feedback pathway. We use field recording and modeling to investigate the interaction between the transient high magnitude potentiation (200-300%) elicited during tetanic stimulation and the lower magnitude post-tetanic potentiation (PTP) (~30%) that slowly decays upon cessation of the tetanus. The amplitude of the observed transient potentiation is graded with stimulus frequency. In contrast, the induction of PTP has a stimulus frequency threshold between 1 Hz and 5 Hz and its amplitude is independent of stimulus frequency. We suggest that the threshold for PTP induction may be linked to a minimum level of sustained potentiation (MSP) during repetitive trains of stimuli. We have developed a novel model that describes the interaction between the transient plasticity observed during train stimulation and the generation of PTP. The model combines a multiplicative, facilitation-depression-type (*FD*) model that describes the transient plasticity, with an enzymatic network that describes the dynamics of PTP. The model links transient plasticity to PTP through an input term that reflects MSP. The stratum fibrosum-pyramidal cell (StF-PC) synapse investigated in this study, is the terminus of a feedback pathway to the electrosensory lateral line lobe (ELL) of a weakly electric gymnotiform fish. Dynamic plasticity at the StF-PC synapse may contribute to the putative role of this feedback pathway as a sensory searchlight.

Introduction

In an ever-changing environment, the primary objective of a sensory system is to distinguish relevant stimuli from a noisy background. The Amazonian, brown ghost knife fish, *Apteronotus leptorhynchus*, relies on its electric sense to navigate a complex environment of organic and inorganic matter, find food and mates, and avoid predation. A wide variety of stimuli, including the animal's own movements, other electric fish and organic and inorganic objects, cause distortion in the fish's electric field.

Electroreceptors on the skin surface respond vigorously to these distortions regardless of whether they are caused by reafferent stimuli such as tail movements or by novel stimuli such as prey (Bastian, 1981a).

Electroreceptor afferents project to the electrosensory lateral line lobe (ELL), a hindbrain structure that serves as the first order processing center for the electric sense. Here they form synapses with the basal dendrites of pyramidal cells (Maler, 1974). In addition to receiving afferent input, ELL pyramidal cells also receive feedback input from higher brain centers onto their apical dendrites (Maler, 1981).

As the major output neurons of the ELL, the pyramidal cells encode input from a population of electroreceptors (Ratnam, 2000; Chacron, 2001) and transmit it to higher brain centers (Bastian, 1981b; Gabbiani, 1996). However, unlike electroreceptors, pyramidal cells respond vigorously only to novel electrosensory input and these responses decay with repeated presentation of the stimulus (Bastian, 1995). Thus, pyramidal cells act as adaptive filters of predictable input.

It has been shown that this adaptive filtering is, in part, dependent on anti-Hebbian synaptic plasticity between the direct feedback pathway that descends from the

nucleus praeminentialis (NPd) via the stratum fibrosum (StF), and the proximal apical dendrites of ELL pyramidal cells (PC) (Bastian, 1996a, 1996b, 1998a). Earlier *in vivo* work has shown that high frequency (70-100 Hz) trains of stimuli delivered to the StF predominantly result in potentiation of the StF-PC synapse (Bastian, 1996b). It has also been shown that the short term form of plasticity, PTP, can be readily elicited at StF-PC synapses *in vitro* and may depend on presynaptic CAMKII α (Bastian, 1996b; Wang, 1997, 1998).

In the present study we identify and characterize multiple forms of short term plasticity including facilitation, short term depression and PTP at StF-PC synapses. We also describe the properties of PTP at these synapses including the stimulation frequency threshold for induction of PTP and the stimulation frequency independent amplitude of PTP. Finally, we have developed a phenomenological model that not only describes the observed transient dynamics of facilitation and depression during train stimulation, but also accounts for both the dynamics and threshold properties of PTP. In doing so we are able to make some suggestions as to the potential role of short term synaptic plasticity in ELL sensory processing.

Methods

Transverse brain slices from the ELL of the brown ghost knife fish, *Apteronotus leptorhynchus*, were prepared according to animal care guidelines established by the University of Ottawa and as previously described (Berman, 1997). Briefly, the fish were anesthetized with MS222 and then respiration with oxygenated water containing MS222. The brain stem was removed, glued to a plastic stage and then immersed in ice cold artificial cerebrospinal fluid [ACSF, composed of (in mM) 124 NaCl, 24 NaHCO₃, 10 D-

Glucose, 1.25 KH_2PO_4 , 2 KCl, 2 MgSO_4 , and 2 CaCl_2 ; all chemicals from Sigma unless otherwise noted]. ELL slices (350 μm) were cut using a Vibraslice and immediately transferred, rostral side up, to the recording chamber. The slices were then maintained at room temperature (20-22°C) in oxygenated ACSF for 1-2 hours prior to recording.

The fibers of the StF are myelinated and appear opaque in relation to the rest of the slice. Thus, the StF is an easily identified landmark for the placement of stimulating and recording electrodes. A monopolar tungsten electrode (<5- μm tip diameter) was positioned in the medial segment (MS) of the ELL at the dorsal aspect of the StF to preferentially stimulate the excitatory afferents which run dorsally and terminate in the ventral molecular layer (VML) (Mathieson, 1988; Berman, 1997). Stimulus timing was computer-controlled (Igor Pro, Wavemetrics Inc, OR; and Pulse Control, (Herrington, 1995), and delivered as 20-40 μs pulses of 20-35 V through a stimulus isolation unit (Digitimer Ltd, Herts, England). Stimulation intensity was determined as that which produced maximal paired pulse facilitation, based on two stimulus pulses delivered 50 ms apart yet remained subthreshold for action potential generation. This permitted measurement of field EPSPs during train stimulation without contamination by action potentials or their after-hyperpolarizations.

Baseline stimulation consisted of single or paired pulses (50 ms apart) delivered at 0.1 Hz for up to 18 minutes. Tetanic stimulation was delivered as 10, 10 pulse, trains with a 1s inter-train interval. Train frequency varied from 1, 5, 50 or 100 Hz. Test pulses (single or paired) were delivered in the 1 s between the trains at 500 ms.

Field potentials were recorded using 3-10 $\text{M}\Omega$ borosilicate glass electrodes, (Brown-Flaming P-87, Sutter Instruments, CA) filled with ACSF and positioned in the

VML of the centromedial segment (CMS). After a stable baseline was obtained, tetanic stimulation was applied and the transient long term effect of the tetanus on EPSP amplitude was assessed by test pulses delivered every 10 s for 15 min starting 5 seconds after the last train.

Data Analysis

Field potentials were amplified and low pass filtered (3 KHz) (Axoclamp 2A, Axon Instruments Inc, Burlingame CA.), digitized (Instrutech Corp, Greatneck, N.Y.), and stored for analysis (Igor Pro and Pulse Control). Analysis was done with IgorPro. Statistical significance was assessed by analysis of variance (ANOVA). All results are reported with ($\pm$) standard error of the mean (SE) unless otherwise stated.

Model Description

In this paper, we discuss two qualitatively distinct forms of short term synaptic enhancement (transient *facilitation*- and *depression*-like processes, on the order of milliseconds and seconds, and a *post-tetanic potentiation*, PTP, on the order of minutes). In this light, our model follows two distinct formalisms. Facilitation and depression are modeled by a previously described formalism for short term synaptic plasticity (Magleby, 1975; Fischer, 1997; Varela, 1997; Dittman, 2000). We refer to this as the *FD* model, and summarize it briefly below. This type of model is sufficient for describing the short-term facilitation and depression in both the direct and indirect feedback pathways to ELL (Lewis, 2000). However, *FD* models are not capable of explaining the combination of short-term and longer term plasticity described in the present study because the long time constants required for the slow decay (of PTP for example) would result in synaptic enhancement under control stimulation conditions (i.e. in the absence of a tetanus).

Therefore a different formalism must be used to explain synaptic enhancement on the longer time scales. We build on the traditional *FD* model by describing PTP with two additional variables, X_P and Y_P , whose dynamics are given by a set of coupled ordinary differential equations similar to those described in enzymatic networks (for example see: Matsushita, 1995).

In the *FD* model, a postsynaptic potential (*PSP*) due to a brief stimulus pulse is given by the combination of a number of processes that can either increase (*F* processes) or decrease (*D* processes) the PSP relative to its control value (A_o). In preliminary analyses we found that 4 different processes (F_1, F_2, D_1, D_2) were required to adequately fit the *FD* model to the PSPs during the stimulus trains. After each stimulus arrives, the values of F_i and D_i change by a discrete amount, the update magnitude (Eq. 1).

$$\begin{aligned} F_1 &\rightarrow f_1 + F_1 \\ F_2 &\rightarrow f_2 + F_2 \\ D_1 &\rightarrow (1 - d_1[F_1 - 1]) \bullet D_1 \\ D_2 &\rightarrow d_2 \bullet D_2 \end{aligned} \tag{Eq. 1}$$

where $\bullet$ represents multiplication. The update magnitude for the D_1 process is related to F_1 , similar to previous studies (Dittman, 2000; Hempel, 2000). This was required to account for the range of responses that were observed across the different frequencies tested. Between stimuli, F_i and D_i decay exponentially to a value of one with their respective time constants (Eq. 2).

$$\begin{aligned} \tau_{F_i} \frac{dF_i}{dt} &= 1 - F_i \\ \tau_{D_i} \frac{dD_i}{dt} &= 1 - D_i \end{aligned} \tag{Eq. 2}$$

The post-train dynamics of PTP were described by a qualitatively different process, F_{PTP} . The variables X_P and Y_P , whose dynamics are described in Eq. 3, dictate the dynamics of F_{PTP} (Eq. 4)

$$\begin{aligned}\tau_X \frac{dX_P}{dt} &= \frac{(w_1 X_P - Y_P)^2}{k^2 + (w_1 X_P - Y_P)^2} - X_P \\ \tau_Y \frac{dY_P}{dt} &= w_2 X_P - Y_P\end{aligned}\tag{Eq. 3}$$

$$F_{PTP} = 1 + w_3 Y_P\tag{Eq. 4}$$

At each stimulus, X_P is perturbed by an amount S (Eq. 5). The process described by S shows dynamics similar to those of F_2 in the current formulation (Eq. 6).

$$X_P \rightarrow S + X_P\tag{Eq. 5}$$

$$\begin{aligned}S &\rightarrow s_o + S \\ \tau_s \frac{dS}{dt} &= -S\end{aligned}\tag{Eq. 6}.$$

The overall responses of the model to stimulation are given by a combination of 5 different processes (Eq. 7), as in previous studies (Fischer, et al. 1997).

$$PSP = A_o \bullet (F_1 + F_2) \bullet D_1 \bullet D_2 \bullet F_{PTP}\tag{Eq. 7}$$

Initially the model was fit to mean 5 Hz and 50 Hz data sequentially and Table 1 shows the list of model parameters and the values that fit these stimulus protocols. An asterisk next to the parameter indicates that the parameter was fitted by least-squares, otherwise parameter values were fixed at constant values. The data from 1 Hz and 100 Hz were not used for this initial fit. With these parameter values (Table 1), the root-mean-squared

(RMS) errors for each of the four frequencies (1, 5, 50, 100 Hz) were 5.4%, 6.1%, 5.4%, and 17.2% respectively. In addition to fitting the mean data, we also performed least-squares fits of the model to data from individual slices where both 50Hz and 100Hz stimulation were performed (see Results). In these fits, most parameters were constrained to the values shown in Table 1. Initially, the 50 Hz data was fit with the free parameters being f_1 , f_2 , d_1 , d_2 , and w_3 . From these fits, it was apparent that the values of f_1 and d_1 had the highest variability; the coefficients of

Table 1. Model parameters.

parameter	value	LS fit
f_1	1.814	*
τF_1	0.0211	*
f_2	0.435	*
τF_2	0.903	*
d_1	0.0567	*
τD_1	1.35	*
d_2	0.995	*
τD_2	8.85	*
k	0.5	
w_1	1.2	
w_2	0.25	
w_3	2.00	*
τ_x	10	
τ_y	130	
s_0	0.004	
τ_s	1.2	

variation for each of the 5 parameters were 0.46, 0.35, 0.54, 0.002, 0.29 respectively. We then tested whether the 100 Hz data could be explained by changes in only f_1 and d_1 by fitting the data from both 50 Hz and 100 Hz stimulation with only these two parameters free to vary, and the remaining three parameters fixed to the values found in the 50 Hz fits. In 6 of 8 cases, varying the values of these two parameters could explain a large part of the differences between the 50 Hz and 100 Hz data (RMS error < 15%). Table 2 shows how these parameters changed between the fits to the 50 Hz and 100Hz data (also see Results).

Table 2. Summary of 50Hz/100Hz comparison.

parameter	mean value	S.D.
50Hz f_1	1.8	0.83
50Hz d_1	0.057	0.031
parameter	mean % diff. from 50Hz value	S.D.
100Hz f_1	37%	41%
100Hz d_1	290%	212%

Results

The direct feedback pathway connects the ELL and the nucleus praeminentialis (Pd) (Figure 1). Briefly, pyramidal cells project axons to the Pd, activating stellate cells

(glutamatergic). The Pd stellate cells then project to the ELL via the stratum fibrosum (StF) and synapse on the spines of the proximal apical dendrites of pyramidal cells.

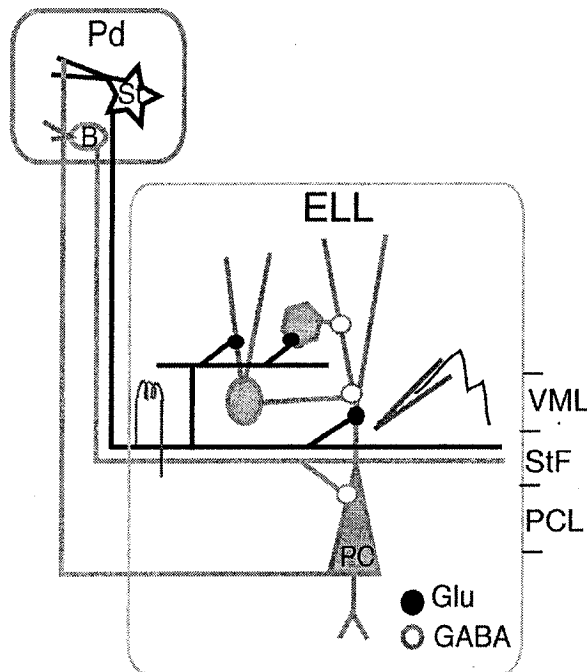


Figure 1: Circuitry of the Direct Feedback Pathway. The direct

feedback circuit is part of a closed loop between the nucleus praeminentialis (Pd) and the electrosensory lateral line lobe (ELL). Pyramidal cell (PC) axons contact Pd stellate cells (St) and bipolar cells (B). The axons of the stellate and bipolar cells form the excitatory and inhibitory tracts, respectively, of the stratum fibrosum (StF). The StF then closes the feedback loop by making excitatory (Glu⁺, glutamate) and inhibitory (GABAergic) connections of the apical dendrites and soma of the PCs. In addition, excitatory fibers also synapse with local ELL interneurons, I, which make inhibitory contacts with PCs. In the slice preparation, the StF was stimulated in the medial segment of the ELL and field recordings were taken in the ventral molecular layer, (VML) of the centromedial segment. PCL, pyramidal cell layer.

Stimulation of the StF evoked field potentials similar to those previously described (Berman, 1997; Wang, 1997). The biphasic field potential had a brief positivity that was often obscured by the fiber volley, followed by a larger negativity with an amplitude of 0.4 - 1.5 mV depending on stimulus intensity; the latency to peak of this negativity was ~5.5 ms (5.45 ± 1.01 ms, mean and standard deviation) (Figure 2A).

Previous studies using current source density (CSD), intracellular recording, and pharmacological analysis have demonstrated that this negativity represents StF-evoked compound EPSPs (Berman, 1997). As in previous studies (Varela, 1997), we utilize field recordings in order to reduce the damage caused by intracellular impalement which allows long term recording at the same site enabling us to compare different stimulation frequencies. We have confirmed our results with a small number of intracellular recordings (n=5, data not shown). Baseline StF stimulation, consisting of single or paired pulses delivered every 10 seconds for up to 18 minutes did not result in potentiation of field EPSPs ($99.11 \pm 2.44\%$). As previously reported (Wang, 1997, 1998), fiber volleys at all stimulation frequencies, either transiently decreased ($< 10\%$) or did not change during the trains. Following the trains, volleys changed slightly ($\pm 5\% \pm 2\%$) or did not change at all. Since the potentiation of EPSP amplitudes is consistent regardless of the small positive or negative changes in fiber volley, it is unlikely that a change in fiber volley contributes significantly to the 20 % to $>300\%$ changes EPSP amplitude. In a few slices (n=4) the fiber volleys increased up to 20% following train stimulation. In these cases, the EPSP was fused to the fiber volley and these measurements were likely contaminated. Consequently, these slices were not included in the present analysis.

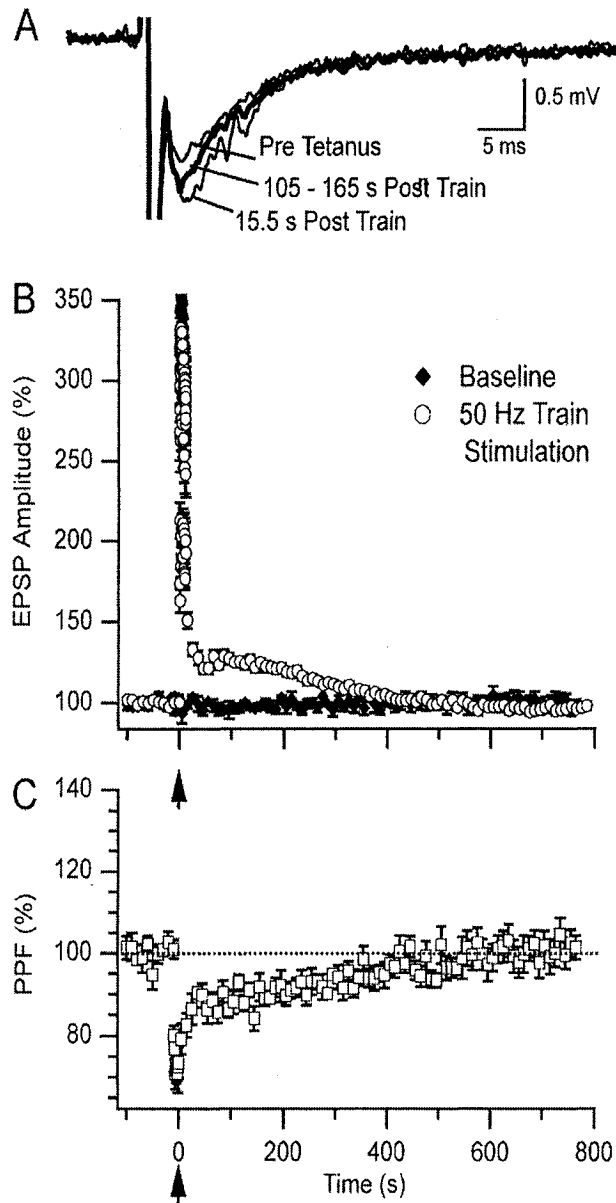


Figure 2: Short-term synaptic plasticity in StF-PC synapses.

Stimulation of the StF with ten, 50 Hz trains (delivered at arrow) resulted in a large transient potentiation during the trains followed by a smaller slowly decaying potentiation upon train cessation. A) Field EPSPs taken from the VML in response to StF stimulation. Pre-tetanus trace: thick black line, average of 10 baseline pulses (0.01 Hz). Post-tetanus traces: thin black line, single trace taken 15.5 s after trains cessation; thick gray trace, average of 5 traces (10 s apart) during stable phase of PTP decay, 105-165 s post tetanus. B) Mean response ($n=19$ slices) to 50 Hz train stimulation. EPSP amplitudes (open circles) are reported as a % of pre-train baseline values, error bars SE. EPSP amplitudes remain significantly different from baseline values (closed diamonds) for up to 260 s following train cessation (*, $p<0.01$) C) Paired pulse facilitation in response to same protocol in B. PPF values are presented as a % of pre-train baseline values. Reduction in PPF is maximal during train stimulation and PPF values return to baseline with a time course similar to PTP.

Potentiation in Response to Train Stimulation at Varying Frequencies

A total of 28 slices were studied. Of these slices, 25 demonstrated short term synaptic potentiation in response to tetanic stimulation without significant change in fiber

volley, 3 were excluded from analysis due to depression during and following train stimulation. Of the 25 slices that potentiated, 19 showed a sustained (~5 min) potentiation following cessation of the tetanus.

Tetanic stimulation consisted of ten, 10 pulse trains of varying frequencies with 1 s between trains. This burst type protocol mimics the *in vivo* firing pattern of the Pd stellate cells which give rise to the StF fibers. Upon excitation, the normally quiet stellate cells produce short bursts of activity that have an average frequency of 80 Hz over a 100 ms period (Bratton, 1990). However, as will be shown in this paper, higher frequencies of train stimulation lead to increasingly variable responses during stimulation; thus, our standard frequency of stimulation during the trains was 50 Hz.

Tetanic stimulation of the StF with 50 Hz trains resulted in a large mean transient increase in EPSP amplitudes during train stimulation ($328 \pm 24\%$) (Figure 2B). This transient potentiation decayed to $151 \pm 5\%$ within 5 seconds of train cessation. Following this, EPSP amplitudes plateaued for ~2 minutes around $124 \pm 3\%$. The EPSPs remained significantly potentiated over baseline values for 4.3 minutes ($p < 0.01$) after train cessation (Figure 2B). The decay from 5 s post tetanus to baseline was fit with a single exponential with a τ value of 6.19 min. This τ value is suggestive of post-tetanic potentiation (PTP) as seen in other systems (Fischer, 1997).

In six experiments single test pulses were replaced with paired pulses, 50 ms apart, to ascertain the possible locus of the potentiation. During baseline stimulation, there was an average paired pulse facilitation (PPF) of $176\% \pm 10\%$. During train stimulation PPF was reduced to $125 \pm 4\%$. Following the trains there was a significant sustained reduction in PPF ($153 \pm 8\%$, 5 min ($p < 0.01$)) that returned to baseline levels

with a similar time course to PTP (Figure 2C). These reductions in PPF suggest that the likely source of both transient potentiation and PTP is a presynaptic increase in synaptic efficacy (for review see Zucker 1989).

In trials where train stimulation was changed to 1 Hz, 5 Hz or 100 Hz, tetanic stimulation of the StF resulted in graded degrees of potentiation during the trains of stimuli that was dependent on stimulation frequency (Figure 3).

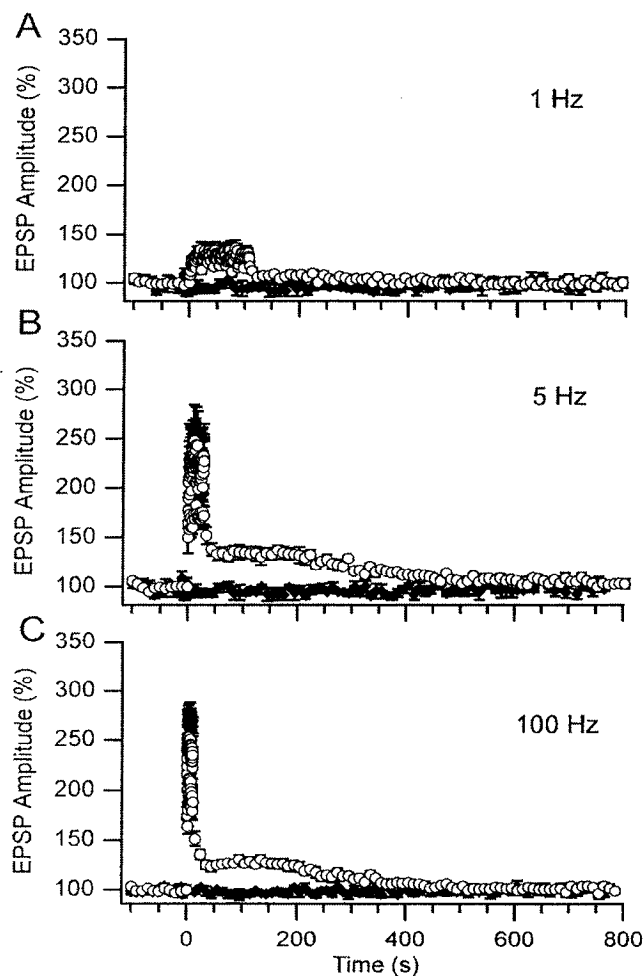


Figure 3: Frequency dependent changes in short term synaptic plasticity at StF-PC synapses. Mean responses to ten, 10-pulse trains of 1 Hz, 5 Hz, 100 Hz during tetanic stimulation. EPSP amplitudes (open circles, mean $\pm$ SE) for all frequencies are reported as % of pre-train baseline values. A) During 1 Hz trains there is minimal transient potentiation ($<140\%$) and no demonstrable PTP following the trains. B) and C) 5 Hz and 100 Hz train stimulation resulted in 200-250% transient potentiation, followed by PTP with amplitude and decay characteristics similar to that reported following 50 Hz trains (Fig 2B).

The mean transient potentiation during the trains had maximum amplitudes of $136 \pm 8\%$ (1 Hz, $n=6$, Fig. 3A) and $200 \pm 20\%$ (5 Hz, $n=7$, Fig. 3B). Interestingly, during the 100

Hz trains, the mean maximum potentiation is less ($231 \pm 36\%$ $n=8$) than that during 50 Hz (Compare Figure 2B and Fig. 3C).

In contrast to the transient potentiation during the trains which was stimulation frequency dependent, 5 Hz and 100 Hz stimulation resulted in a level of PTP comparable to that seen at 50 Hz suggesting the amplitude of PTP is stimulation frequency independent. The maximum post train amplitudes (at 5 s) were $152 \pm 6\%$ (5 Hz) and $151 \pm 5\%$ (100 Hz). The amplitudes of the plateau potentiations were $133 \pm 6\%$ (5 Hz) and $126 \pm 4\%$ (100 Hz) (Figure 3 B and C). These amplitudes were not significantly different from those seen with 50 Hz stimulation. The τ -values of PTP decay were 6.81 min (5 Hz) and 5.49 min (100 Hz). Like the results seen at 50 Hz, both 5 Hz and 100 Hz stimulation resulted in decreased PPF during the trains as well as sustained reduction in PPF following the trains (data not shown).

It is important to note that 1 Hz train stimulation did not produce PTP as neither the maximum amplitude ($112 \pm 5\%$) nor the average amplitude ($105 \pm 3\%$) following the trains was significantly different from baseline values (maximum: $105 \pm 5\%$, average $98 \pm 3\%$) (Figure 3A). Nor was there a significant reduction in PPF following train cessation (pre-train: $165 \pm 5\%$, post-train: $159 \pm 5\%$).

Determining the PTP Data Set

In a small number of slices (6) train stimulation at 50 Hz resulted in transient potentiation during the trains but failed to produce long lasting PTP (Figure 4A). The mean maximum amplitude at 5 s post train was $112 \pm 7\%$ and the mean plateau amplitude was $108 \pm 5\%$. PPF decreased by 30 % during the trains but only demonstrated a significant reduction in PPF at 5 s post tetanus ($p<0.01$) (Figure 4B).

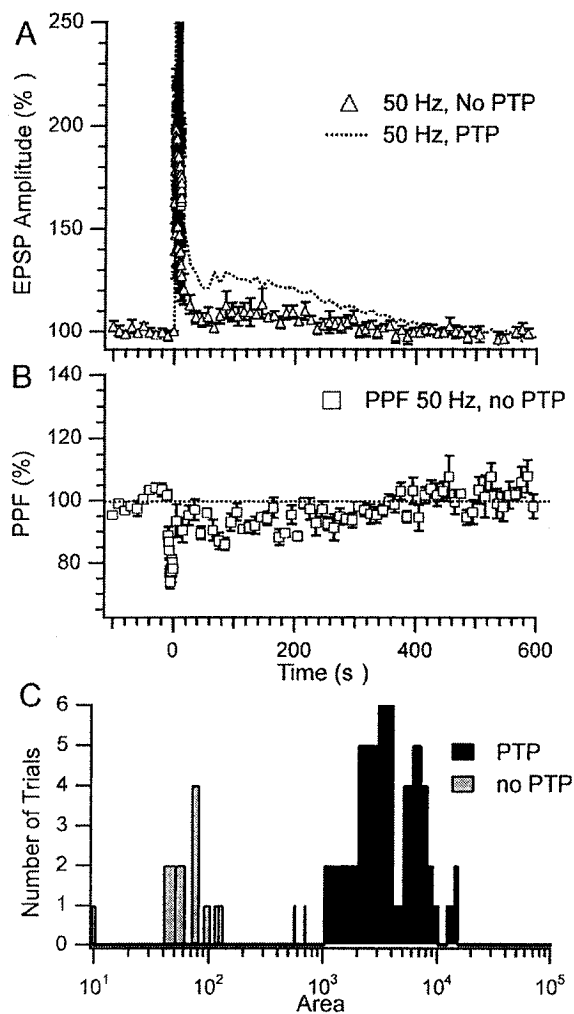


Figure 4: Comparison of PTP

trials versus no PTP trials. A) In six slices, 50 Hz stimulation did not yield PTP (open triangles). Dotted line represents mean PTP in response to 50 Hz stimulation. B) PPF following the trains in non-PTP trials was not significantly different from baseline values. C) Areas under the decay curves following train cessation were assessed in all trials. Briefly, the decay curves were smoothed from 5 s post trains to 720 s. The area was then measured to the point where the decay curve first crossed 109% (established by baseline variation). A bimodal distribution of the data set is evident with trials that demonstrated PTP (most 5 Hz and 50 Hz data) (filled bars, areas >1000) and trials (a few trials at 5 Hz and 50 Hz, as well as all 1 Hz data) that did not demonstrate PTP, "no PTP" (shaded bars, areas ≤ 100).

These results, in conjunction with the 1 Hz data set suggest that quantitative criteria are required to distinguish slices in which PTP is elicited from those where it is not. We developed a procedure to quantify PTP following the trains; a similar method has been described previously (Jensen, et al. 1999). This procedure takes into account both the duration and amplitude of PTP. The first step was to establish a point when the potentiation would be considered recovered. The baseline EPSP amplitudes are normalized to 100% and vary with a standard deviation of 9 %. Thus, we set 109 % as our "recovery amplitude". We then found the first point at which the smoothed decay

curve crossed 109% and set that time point as "recovered". We then took the area under the decay curve from 5 s post train to the "recovered" time point. This resulted in a bimodal data set of "PTP" versus "no PTP" trials, where PTP trials had areas of 1000 or greater and "no PTP" trials had areas less than 100 (Fig. 4C). Only slices that had areas that met the "PTP" criteria were used for analysis and modeling.

Minimum Sustained Potentiation

In comparing the two data sets "PTP" and "no PTP", as defined by our assessment of area under the decay curve (Fig. 4C), there appears to be a minimum level of potentiation sustained during train stimulation in the "PTP" set that is not sustained in the "no PTP" set. In order to determine a value for this minimum sustained potentiation (MSP), the sustained potentiation in each trial was quantified and the MSP for the PTP data set was ascertained. Briefly, the EPSP amplitude at the first pulse of each train (with the exception of the first train) is the remaining potentiation from the previous train summed with the current stimulus. This serves to estimate the level of potentiation sustained between trains. Thus, for each individual trial the first pulse of each train (excluding the first) was measured. These values were then averaged and the mean value represents the sustained potentiation for that trial. In all cases that demonstrate PTP (trains of 5 Hz-100 Hz) the sustained potentiation during train stimulation is at least $150 \pm 6\%$ (determined as the mean sustained potentiation during 5 Hz trains). It is possible that this level may indicate threshold for PTP generation since the sustained potentiation during 1 Hz trains (no PTP) is $122 \pm 4\%$, and only $127 \pm 3\%$ in non PTP trials of higher frequencies. Both of these values are significantly different from sustained potentiation at 5 Hz ($p < 0.05$) and 50 Hz (MSP = $170 \pm 4\%$) ($P < 0.01$) (Figure 5A). We suggest that it

is the *minimum* sustained potentiation during the trains that must surpass a threshold for PTP rather than the maximum potentiation (measured as the mean of the peak amplitudes of each train) for two reasons. First, the mean maximum potentiation at 5 Hz ($200 \pm 20\%$) is significantly less ($p < 0.01$) than 50 Hz ($328 \pm 24\%$) but 5 Hz stimulation still elicits PTP. Second the mean maximum potentiation of the 5 Hz set is not significantly different than that of the "no PTP" set ($179 \pm 13\%$), yet 5 Hz stimulation yields PTP while the latter does not (Figure 5B). When sustained potentiation is plotted against the area under the PTP decay curve there is a distinct clustering of points for the "PTP" and "no PTP" trials (Figure 5C). These results suggest that the MSP is related to the threshold for the induction of PTP.

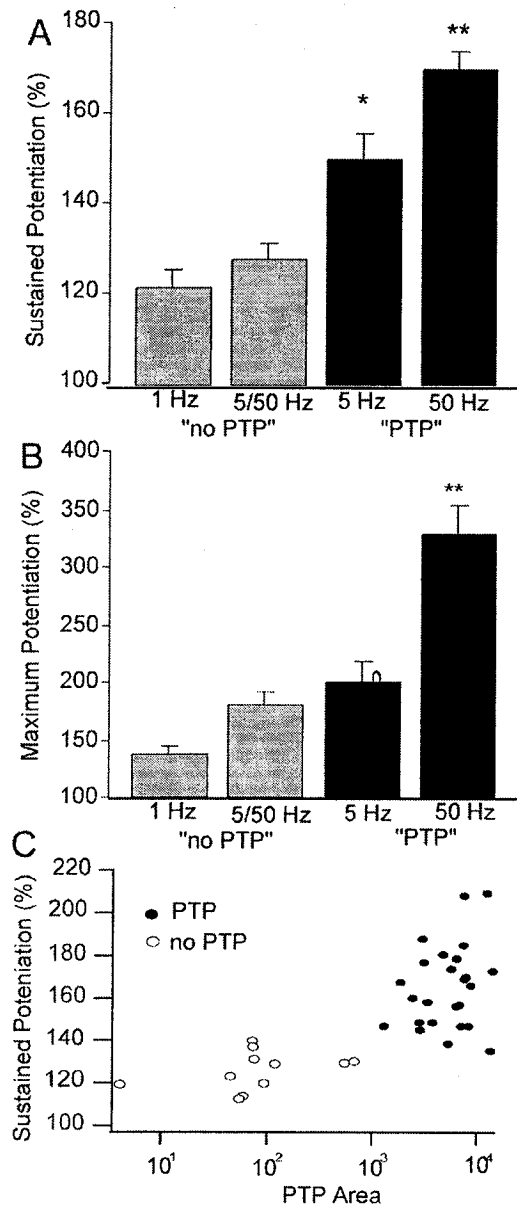


Figure 5: Minimum Sustained Potentiation (MSP) during train stimulation.

A) The sustained potentiation (%) during train stimulation measured as the mean of the amplitudes of the first EPSP of each train (except the first train). The sustained potentiation in the "no PTP" trials (gray bars, 1 Hz, 5 Hz/50 Hz "no PTP") was significantly less than "PTP" trials (black bars, 5 Hz and 50 Hz "PTP") (error bars: SE). The sustained potentiation during 50 Hz stimulation was significantly greater than 5 Hz ($p < 0.05$) and the no PTP trials (**, $p < 0.01$). The sustained potentiation during the 5 Hz "PTP" trials was significantly greater than the "no PTP" trials (*, $p < 0.05$) suggesting the MSP for PTP induction is at least 150%. **B)** Maximum potentiation (%) (mean of the peak EPSP amplitudes of each train). Only 50 Hz stimulation during the PTP trials demonstrated maximum potentiation that was significantly different from the other trials (**, $p < 0.01$). **C)** Sustained potentiation (%) plotted against the areas under the decay curves for "PTP" (closed circles) and "no PTP" (open circles) trials. There is a distinct clustering of points for the "PTP" and "no PTP" cases.

Modeling Short Term Enhancement in the ELL

In looking at the EPSP amplitudes attained during 50 Hz train stimulation, it is evident that multiple processes contribute to StF-pyramidal cell (PC) plasticity (shown schematically in Figure 6).

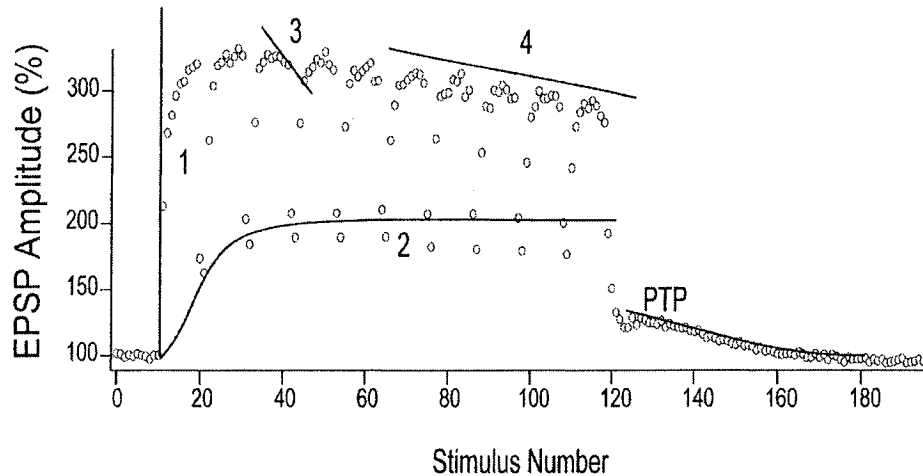


Figure 6: Schematic of Plasticity at the StF-PC Synapse with 50 Hz Stimulation.

EPSP amplitude versus stimulus number for the mean 50 Hz data set. Five trends are apparent and shown schematically. During the trains, there appear to be two potentiating trends; one which is maximal within the first few pulses of each train (1) and another which builds more slowly and is sustained across the trains.(2) There also appear to be two trends that decrease potentiation: one that decreases EPSP amplitudes during individual trains (3) and a second which builds during later trains (4). Finally a fifth trend (PTP), decays slowly upon cessation of train stimulation.

During train stimulation, two facilitation processes are apparent. The first has a fast onset that rapidly increases EPSP amplitude within the first 2 -3 stimuli of each individual train. The second process builds slowly and seems to plateau by the third train. There also appear to be two depression processes; a fast depression that reduces EPSP amplitudes during the last few stimuli of individual trains, and a slower one that becomes

apparent during the later trains of the tetanus. Following train stimulation, at least one more process, PTP is evident.

In order to describe the rapid kinetics of the transient potentiation during train stimulation as well as the more complex dynamics of PTP, we developed a phenomenological model that combines two approaches: we used the previously proposed facilitation and depression (*FD*) models (Magleby, 1975; Varela, 1997; Dittman, 2000; Lewis, 2000) together with coupled differential equations that describe enzymatic networks (Matsushita, 1995). The model comprises five processes and is summarized in the following equation (for model details see Methods):

$$PSP=A_o \cdot (F_1+F_2) \cdot D_1 \cdot D_2 \cdot F_{PTP}$$

In this model, the amplitude (PSP) of the EPSP due to a stimulus is the product of processes that either increase EPSP amplitude (F_1+F_2 , F_{PTP}) or decrease the EPSP amplitude (D_1 , D_2). A_o refers to the baseline EPSP amplitude prior to tetanic stimulation and, up until now, has been presented as 100%. However, in further discussion of the model, all EPSP data will be normalized to a baseline value of 1, thus, this term (A_o) can be omitted. Generally, the contribution of each process in an *FD* model (F_1 , F_2 , D_1 , D_2) to the PSP is dependent on the timing of the stimuli, the decay constant for each process as well as the incremental increase/decrease of each process with stimulation (referred to here as the update magnitude) (Eq. 1, Methods). However, in the case of D_1 , the incremental decrease is linked to the F_1 process. A number of *FD* models link fast depression terms to fast facilitation (Dittman, 2000; Hempel, 2000). In these cases, fast

depression is due to vesicle depletion which can be accelerated by facilitation depending on the probability of release at the synapse. While the probability of release at the StF synapse is unknown, we have found that the frequency dependent potentiation during the trains is also best fit by linking D_1 to F_1 (Eq.1 and 2, Methods).

Since the 5 Hz and 50 Hz data sets were more consistent than the 100 Hz data, the model was initially fit to mean 5 Hz and 50 Hz data sequentially to yield parameter values for each plasticity process. All values resulting from this fit are presented in Table 1. The τ values of the fit to mean 5 Hz and 50 Hz data were then fixed and the update magnitudes were allowed to vary while model was re-fit to all individual data sets at all stimulation frequencies. Though all simulations include all five plasticity processes, the following summary presents model simulation results in two sections, *Transient Potentiation during train stimulation* and *PTP during and following train stimulation*.

Transient Potentiation During Train Stimulation

Simulations of pyramidal cell responses to train input suggest that the majority of the potentiation could be attributed to the contributions of the first four processes. Figure 7 shows the model performance compared to the mean train data at all frequencies tested (parameter values in Table 1).

For the 1 Hz data, the model adequately simulated the nominal increases in EPSP amplitudes (Figure 7A). At 5 and 50 Hz, the model nearly perfectly simulated the mean EPSP amplitudes obtained experimentally (Figure 7B and C). However, the model overestimates the average amount of potentiation in response to 100 Hz train stimulation

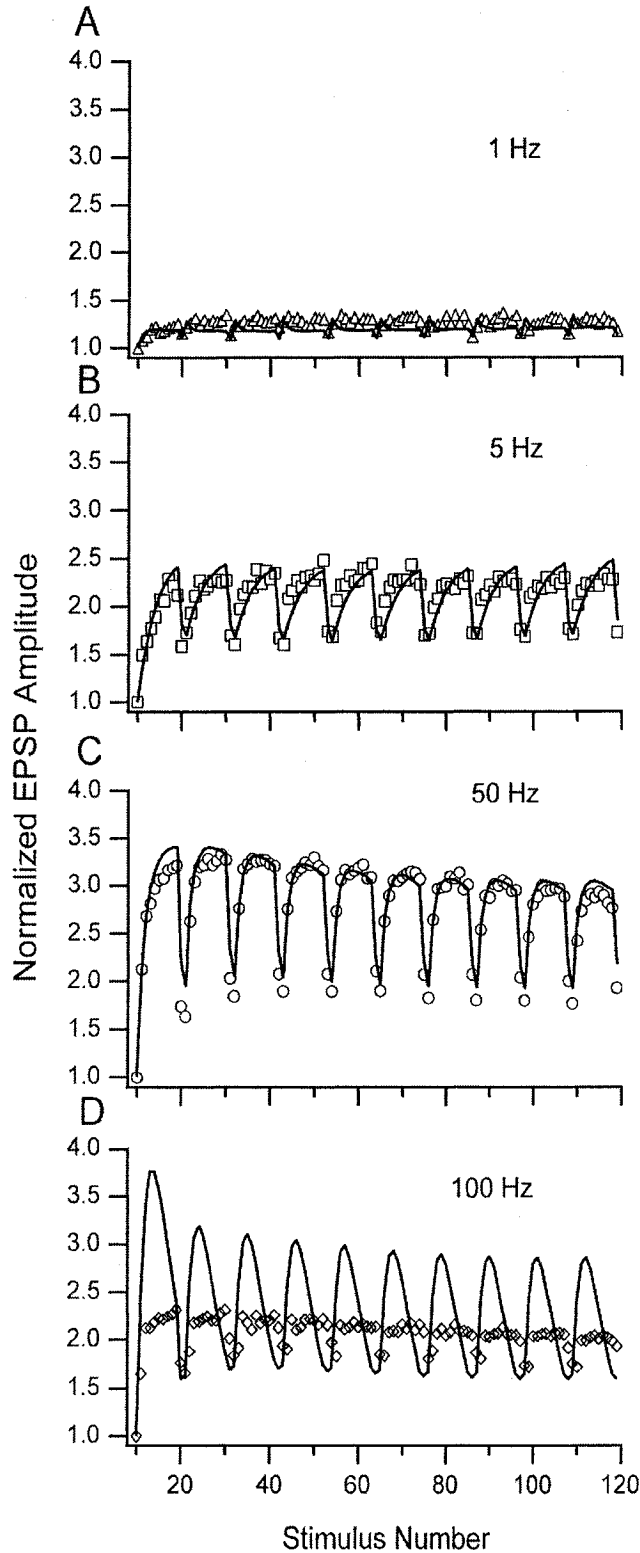


Figure 7: Experimental and simulated transient potentiation during train stimulation. Experimental EPSP amplitudes (mean data, open symbols) during A) 1 Hz, B) 5 Hz, C) 50 Hz, D) 100 Hz trains, plotted against stimulus number and normalized to the first EPSP of the first train. Solid lines represent the simulated EPSP amplitudes based on the $(F_1 + F_2)D_1D_2F_{PTP}$ model. Note that although the responses are discrete, a continuous line is used for clarity (a similar convention is used for all other figures showing model simulations). The τ -values and update magnitudes for each frequency are reported in Table 1. RMS errors for each frequency are 5.4% (1 Hz), 6.1% (5 Hz), 5.4% (50 Hz) and 17.2% (100 Hz).

(Figure 7D). It is possible that the variability seen in the 100 Hz data set could bias the mean and could thus, affect the interpretation of this comparison. In the next section, we investigate this possibility further.

Higher frequency stimulation produces variable results during train stimulation.

To better assess the variable levels of potentiation observed during 100 Hz stimulation we analyzed experiments that directly compare 50 and 100 Hz stimulation in the same slice (n=8). In comparing the mean EPSP amplitudes during 50 Hz and 100 Hz stimulation (Figure 8A) it is apparent that on average, 100 Hz stimulation results in less potentiation than 50 Hz stimulation (hatched circles, Figure 8B). When the maximum potentiation (mean of the peak potentiations in each of the 10 trains) during tetanus was measured in individual experiments, it was found that in 5 of 8 slices, potentiation during 100 Hz tetanic stimulation was less than that seen with 50 Hz (Figure 8B, solid lines). Of the remaining 3 slices, 2 showed potentiation with 100 Hz trains slightly greater than that seen at 50 Hz, while 1 slice demonstrated far greater potentiation during 100 Hz stimulation than 50 Hz stimulation (Figure 8B, dotted lines).

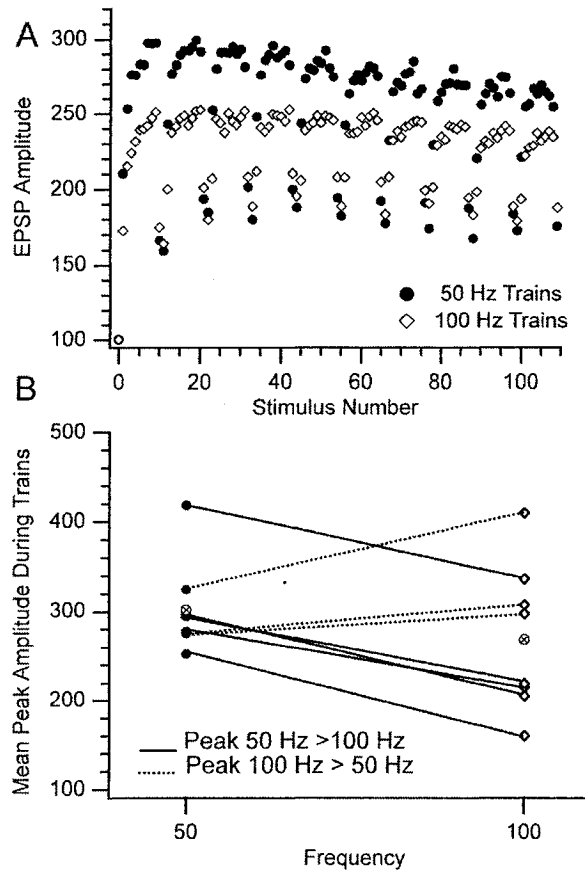


Figure 8: A slice by slice comparison of 50 Hz versus 100 Hz train stimulation. A) The mean EPSP amplitude (% baseline) during 50 Hz trains (closed circles) and 100 Hz trains (open diamonds) are plotted against stimulus number. B) A comparison of the maximum amplitude (% baseline) obtained during 50 Hz (closed circles) and 100 Hz (open diamonds) train stimulation in the same slice. In 5 slices 100 Hz stimulation resulted in a higher maximum amplitude than 50 Hz (solid lines) and in 3 slices 100 Hz potentiation resulted in lower maximum amplitude (dotted lines). The mean values are also plotted (hatch circles) and, as seen in (A), the mean maximum for 100 Hz is less than 50 Hz. Note the broader range of values obtained during 100 Hz stimulation

The variable and unpredictable results observed with 100 Hz stimulation provided an interesting challenge to the model. As seen in figure 7, the model poorly simulates the mean 100 Hz data set. However, by fixing the τ values of F_1 , F_2 , D_1 and D_2 and leaving the update magnitudes (f_1, f_2, d_1, d_2) free, the model was able to fit the individual data sets for both 50 Hz and 100 Hz. In comparing the values of the increments, it was found that in the 100 Hz data sets, f_1 and d_1 departed the most from mean 50 Hz values (Table 2). To test whether the variation in the f_1 and d_1 values was sufficient to explain the variation in the 100 Hz data set, we fixed all τ -values (Table 1), we set f_2 and d_2 to mean values for both frequencies and left f_1 and d_1 free. We then re-fit the individual 50 Hz and 100 Hz

data sets (see Methods). Figure 9 shows the experimental results of two slices illustrating the qualitatively different responses to 50 Hz and 100 Hz stimulation.

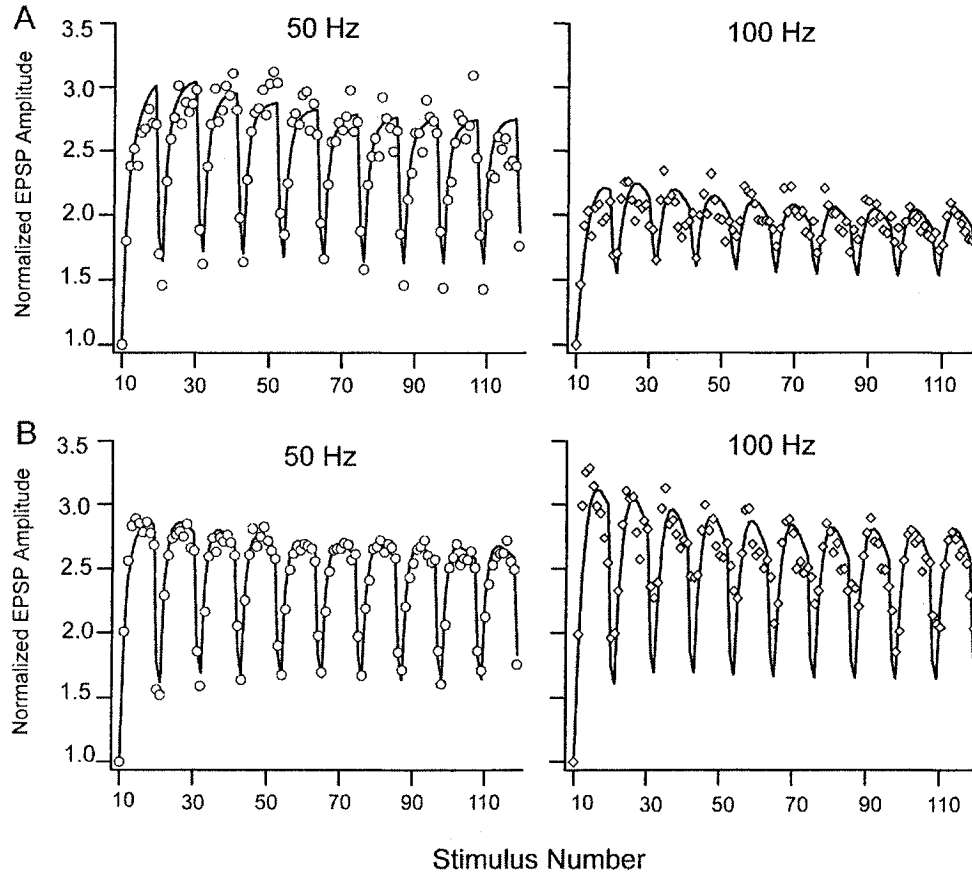


Figure 9: The variable results of individual slices in response to 100 Hz stimulation.

EPSP amplitudes during 50 Hz (open circles) and 100 Hz (open diamonds) train stimulation in two different slices are plotted against stimulus number. The solid lines represent model simulations. In order to achieve the fits, the τ values were fixed and the update magnitudes were allowed to vary. A) 50 Hz stimulation resulted in greater potentiation than 100 Hz stimulation. Fits: τ -values: Table 1, $f_2=0.281$, $d_2=0.9956$, $w_3=1.985$ for both 50Hz and 100Hz and $f_1=1.55$, $d_1=0.0439$ (50 Hz) and $f_1=0.212$, $d_1=0.221$ (100 Hz). RMS errors are 6.6% and 8.0% for 50Hz and 100Hz respectively. B) 100 Hz stimulation resulted in greater potentiation than 50 Hz. Fits: τ -values: Table 1, $f_2=0.292$, $d_2=0.9968$, $w_3=1.638$ for both 50Hz and 100Hz and $f_1=1.74$, $d_1=0.054$ (50 Hz) and $f_1=0.95$, $d_1=0.0488$ (100 Hz). RMS errors are 5.7% and 9.7% for 50Hz and 100Hz respectively.

In both slices, as well as in 4 of the remaining 6 slices, the model was able to adequately describe the experimental results (RMS error <15%). This suggests that the

variation seen at 100 Hz can be explained by a change in the gain of the underlying mechanisms of F_1 and D_1 . However, further work will be required to determine the source of this variation.

PTP During and Following Train Stimulation

The long time constant required to describe the decay of PTP observed ($\tau \cong 6$ min), prohibits its description by an FD model-type process because it would result in synaptic enhancement with baseline stimulation. This is not seen experimentally (baseline: $99.11 \pm 2.44\%$), and further, PTP is not elicited by 1 Hz trains but maximally elicited by trains above 5 Hz. Hence, the model should account for both the frequency threshold of PTP induction during train stimulation as well as the frequency independence of the amplitude of PTP following train cessation.

Both requirements for describing the dynamics of PTP can be achieved using a set of coupled differential equations that are similar to those used to describe enzymatic networks (see Methods). The network is driven by a stimulus term, S , which links the induction of PTP to the amount of enhancement sustained during train stimulation (MSP). These coupled first order differential equations represent the simplest class of model capable of accounting for both the threshold and constant amplitude of PTP.

Previous studies have localized calcium-calmodulin kinase $\text{II}\alpha$ (CAMKII α) to StF fibers, and verified that StF evoked PTP can be blocked by the CAMKII α inhibitor, KN-62 (Wang, 1998; Maler, 1999). CAMKII α is a multimeric enzyme that is able to autophosphorylate its individual subunits and remain active following the decay of a calcium signal (Hanson, 1992). Autophosphorylation is a cooperative, frequency dependent process. Calcium pulses must initially arrive close enough together for the

cooperative binding of calcium calmodulin to the kinase to occur and for autophosphorylation to occur in excess of dephosphorylation by phosphatases (Hanson, 1994). Our simplified enzymatic network is based on an interactive activation (X_p) and deactivation (Y_p) relationship that would be suggested by the interaction between CAMKII α and a phosphatase. We have added the assumption that X_p is able to autoactivate in a manner similar to CAMKII α autophosphorylation. When X_p receives the stimulus-related signal (S) from an external source, the activation of X_p results in the autoactivation of X_p as well as the forward activation of Y_p . Once activated, Y_p negatively feeds back to deactivate X_p . The dynamics of X_p and Y_p were modeled using coupled differential equations (see Eq. 3 and 4, Methods for details).

Characteristics of S: The link between transient potentiation and PTP

The requirements for minimum sustained potentiation (MSP) during the trains have already been described. It is possible that at least one of the transient potentiation processes, F_1 or F_2 , is responsible for producing MSP that surpasses the threshold for PTP induction. It has been shown that a minimum level of potentiation must be sustained between trains for the induction of PTP. Since the τ -decay for F_1 is extremely short (21 ms), the incremental increase in EPSP amplitude due to F_1 decays completely during the 1 s intervals between the trains; thus, F_1 cannot be a source of sustained potentiation. However, F_2 has a much longer τ , (903 ms), and could therefore, contribute significantly to sustained potentiation between test pulses. However, although F_2 may be correlated with PTP, we do not have any direct evidence that F_2 is a causal factor in producing PTP. Therefore, S is described by an FD -type process with dynamics comparable to those of F_2 . (Eq. 5 and 6, Methods)

The model adequately describes PTP at all frequencies (1-100 Hz) except for the first few points following the trains, which are underestimated (Figure 10). During this time, there is a dip in simulated amplitudes due to the fast offset of F_1 and F_2 compounded with the longer lasting effects of D_2 . However, this underestimation is carried on somewhat longer at 5 Hz (Figure 10B). This may be because the experimental EPSP amplitudes during PTP following 5 Hz stimulation were slightly higher than following 50 or 100 Hz stimulation, whereas the model shows a PTP amplitude that is slightly less following 5 Hz stimulation. Alternatively, a fourth short term, post-tetanic enhancement process, augmentation, might be required to explain this data. Augmentation typically has a decay constant in the 10 s - 1 min range, and has also been attributed to residual calcium (Fischer et al. 1997). The 170 % decay that occurs over the course of the first 15 s following a 50 Hz tetanus is in direct contrast to the rapid decay predicted by model simulations. This suggests that incorporating an augmentation process, with a τ value intermediary of F_2 and F_{PTP} , into the model may account for the underestimation of potentiation directly following the trains. Our protocol, which delivers test pulses 10 s apart following train stimulation, does not enable us to adequately assess the fast decay of a putative augmentation process following the tetanus. We therefore, in the interest of simplicity, did not include an augmentation process into our model.

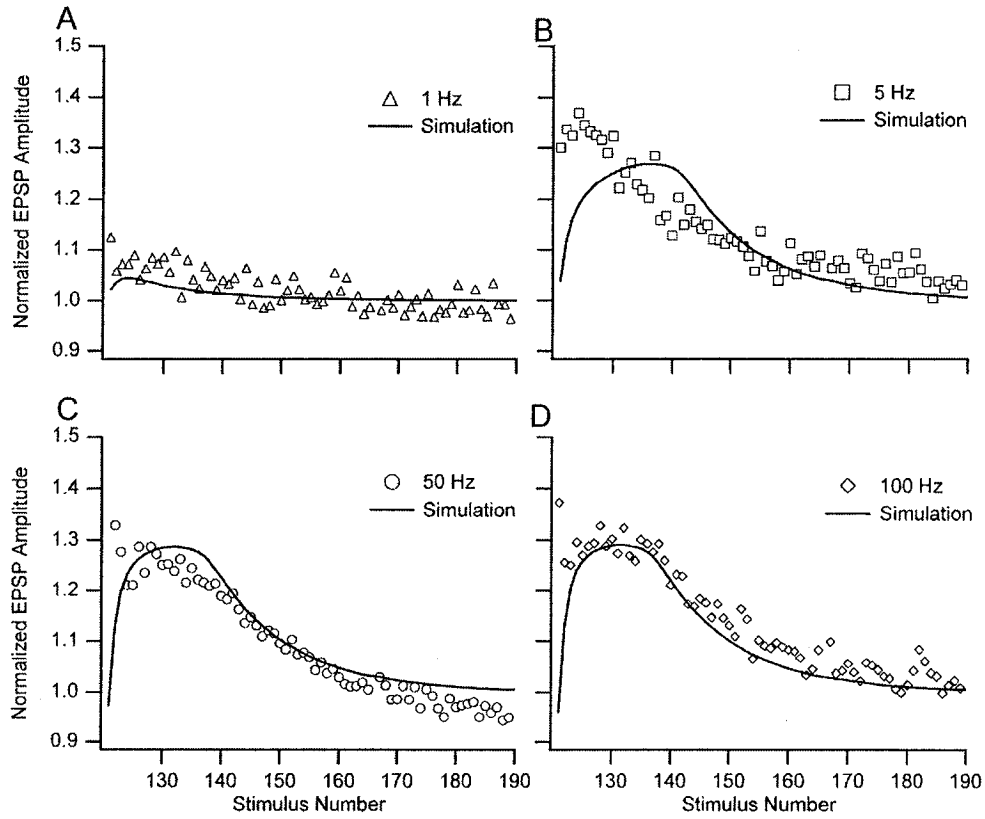


Figure 10: Experimental and simulated PTP following train cessation. Experimental EPSP amplitudes during the decay of PTP following A) 1 Hz, B) 5 Hz, C) 50 Hz, D) 100 Hz trains, plotted against stimulus number and normalized to the pre-train baseline values. Solid lines are the simulated EPSP amplitudes based on the $(F_1+F_2)D_1D_2F_{PTP}$ model (see Table 1 for τ -values and update magnitudes). The model adequately describes the amplitude and time course of PTP following all frequencies of stimulation with the exception of the first few points upon train cessation.

Figure 11 summarizes the model for both STP and PTP as compared to mean experimental results from 5 Hz and 50 Hz stimulation. It also shows the relative contribution of each plasticity process modeled (F_1 , F_2 , D_1 , D_2 , F_{PTP}) at each stimulus (Figure 11 B). Note that F_1 and D_1 contribute relatively little to the amplitudes observed during the trains at 5 Hz, but make significant contributions at 50 Hz. The recruitment of these processes at different frequencies may have implications for the function of feedback over the short duration bursts of activity produced by the stellate cells.

However, also note the slow onset of PTP during the trains and that the contribution of F_{PTP} remains relatively constant regardless of frequency (above 5 Hz).

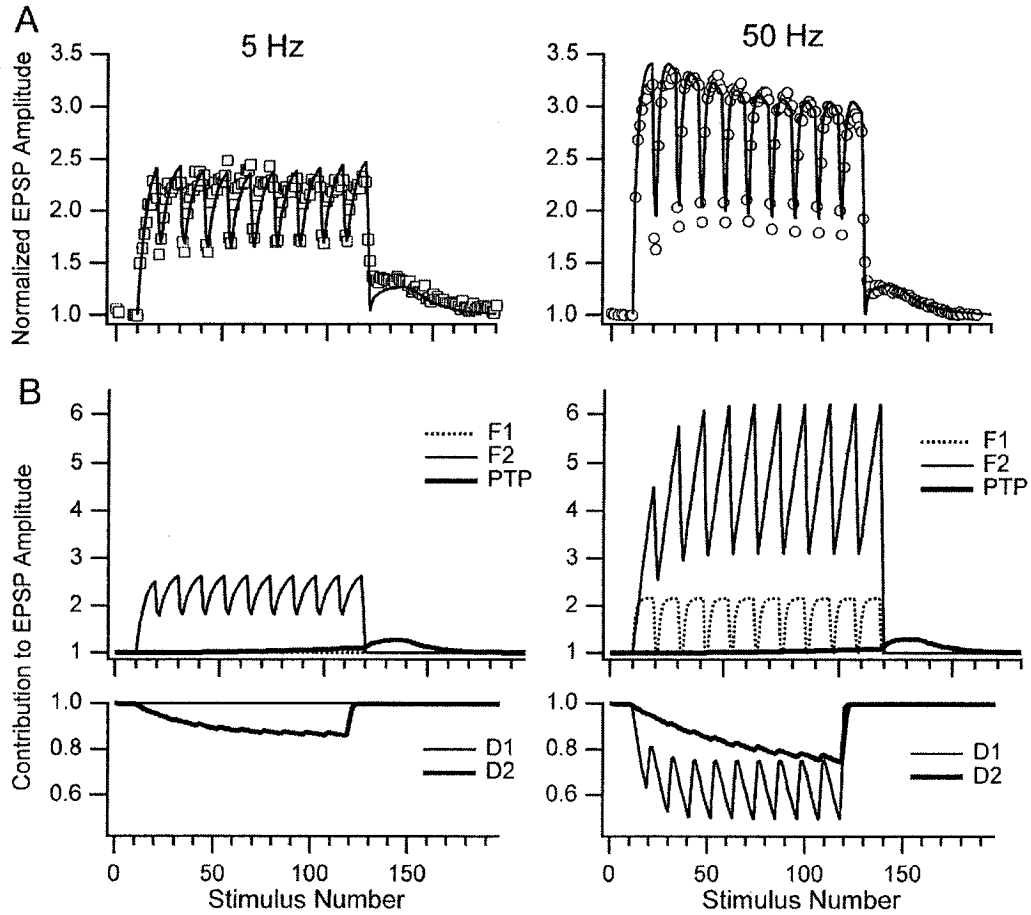


Figure 11: Summary of experimental and simulated results for 5 Hz and 50 Hz

train stimulation. A) Mean experimental EPSP amplitudes (open symbols) to 5 Hz, 50 Hz stimulation plotted against stimulus number. Simulated fits (solid lines) adequately account for both the frequency dependent, high amplitude transient potentiation during trains as well as the frequency independent, low amplitude decay of PTP following the trains. B) The relative contributions of each variable in the $(F_1 + F_2)D_1D_2F_{PTP}$ model to the EPSP amplitude at each point. Note that F_1 and D_1 contribute little during 5 Hz trains but significantly at 50 Hz. Also note that F_2 is the main contributor to EPSP amplitudes during train stimulation.

Discussion

This study demonstrates that tetanic stimulation of the StF results in a large, transient potentiation of EPSP amplitudes during train stimulation. The amplitude of this potentiation was frequency dependent between 1 Hz and 100 Hz, and increased EPSP amplitudes up to three fold at the optimal frequency tested (50 Hz). Following the trains, a smaller, slowly decaying potentiation was observed and referred to as PTP because its decay constant (~ 6 min) is comparable to those reported for PTP in other systems (Fischer et al. 1997). The induction of PTP has a frequency threshold between 1 and 5 Hz but the amplitude of PTP is frequency independent. Experimental evidence suggests that surpassing the threshold for PTP induction depends on the minimum sustained potentiation (MSP) during the trains. Paired pulse analysis suggests that the locus of both transient potentiation and PTP is presynaptic.

We have developed a model that describes both the transient potentiation during the trains and the slowly decaying PTP following the trains. Based on the results obtained both experimentally and through simulations, we suggest that at least five overlapping processes contribute to synaptic plasticity at the StF-Pyramidal cell synapse.

Short Term Processes During Train Stimulation- Facilitation

The high amplitude transient potentiation during the trains is frequency dependent and can be primarily attributed to short-term facilitation (F_1 , F_2). The τ values for F_1 (21 ms) and F_2 (903 ms) are comparable to values for fast decaying facilitation (F_1) and slow decaying facilitation (F_2) in other systems (Fischer et al. 1997). Therefore, given the putative presynaptic locus, the enhancement of EPSP amplitudes during F_1 and F_2

facilitation is most likely due to residual calcium in the presynaptic terminal (Zucker, 1989; Delaney, 1994).

Short Term Processes During Train Stimulation-Depression

There are also two depression-like processes apparent during train stimulation, D_1 and D_2 . The fast depression described by D_1 could be due to vesicle depletion, which, in a number of systems, is a source of short-term depression with a decay constant of 1-2 s (Dittman, 1998; Varela, 1999). The extent of vesicle depletion depends on the probability of release at a given synapse, and often, the interplay between depletion, short-term facilitation, and/or the decay of residual calcium in the nerve terminal. Though these parameters have not been established in the ELL, vesicle depletion remains a possible mechanism for D_1 .

Alternatively, D_1 may be due to disynaptic GABAergic inhibition. StF fibers make direct excitatory feedback connections onto GABAergic interneurons which synapse onto the apical dendrites and bodies of pyramidal cells. The StF-interneuron synapse could be potentiated in a manner similar to StF-PC synapses resulting in enhanced inhibition through the interneuron -PC synapses (Maler, 1981; Berman, 1998b).

The longer time constant of D_2 suggests two other possibilities. Previous studies have demonstrated frequency dependent, direct inhibitory feedback through the StF to pyramidal cells. These GABA_B IPSPs last up to 800 ms, and reach peak amplitudes following 100 Hz train stimulation (Berman, 1998a). Alternatively, in *in vivo* experiments where ELL pyramidal cells are loaded with a calcium chelator, train stimulation of the StF results in significantly greater potentiation than that seen in unloaded cells (Bastian, 1998b, 1999). This suggests that there may be calcium-

dependent, post-synaptic depression at this synapse, similar to that which has been demonstrated in the mormyrid ELL (Han, 2000).

Short Term Enhancement –PTP

Following train stimulation, PTP decays over a 6-7 minute period and is of much lower amplitude (20-30% enhancement) than the transient potentiation. The amplitude of PTP is similar to the amplitude of potentiation reported in previous *in vitro* (Wang and Maler 1997, 1998) and *in vivo* experiments (Bastian 1998a). The induction of PTP has a frequency threshold between 1 and 5 Hz. However, the lack of frequency dependence in the amplitude and decay constant of PTP is distinct from other systems, such as the neuromuscular junction, where these values increase with the frequency and duration of the stimulus (Fischer, et al. 1997; Magleby and Zengel 1975).

In cultured hippocampal neurons, the amplitude of PTP of GABAergic IPSCs has been shown to be frequency independent above 5 Hz (Jensen, 1999). Furthermore, similar to our results, this study demonstrated a frequency threshold for the induction of PTP between 2.5 and 5 Hz. These results suggest that the characteristics of PTP in the ELL (frequency threshold, and frequency independent amplitude), may be found in other brain regions.

The Threshold for PTP- a Possible Role for CAMKII α

The mechanism of the frequency threshold is unknown. According to PPF analysis, PTP has a presynaptic locus. It has been shown that PTP is linearly correlated with the decay of presynaptic residual calcium but cannot be explained solely by this decay suggesting that calcium may be acting at a secondary locus (Delaney, 1989). We

propose that presynaptic residual calcium may be activating an enzyme such as CAMKII α .

In the ELL, CAMKII α has been localized to StF fibers (Maler and Hincke 1999) and StF induced PTP is blocked by the bath application CAMKII α inhibitor, KN-62 (Wang and Maler 1998). Previous *in vitro* experiments demonstrate CAMKII α autophosphorylation in response to calcium pulses delivered at frequencies as low as 4 Hz, leading to nearly maximal autonomous levels of kinase activity (De Koninck, 1998). Interestingly, 1 Hz pulses did not result in significant amounts of autophosphorylation or autonomous activity. These results attractively parallel the frequency dependence for PTP induction observed in this study.

Autophosphorylation of CAMKII α is a calcium dependent cooperative process (Hanson, 1992), this suggests that a minimum level of calcium must be sustained during tetanic stimulation to induce autonomous activity and, by extension, changes in synaptic efficacy. Though the calcium dynamics of ELL synapses are unknown, the level of transient enhancement during the trains, which can be mainly attributed to residual calcium, might serve as an indirect indicator of the calcium level in the presynaptic boutons.

MSP, the Link Between Transient Potentiation and PTP

The results of this paper suggest that a minimum sustained potentiation (MSP) during train stimulation is required for PTP induction. This is based on three observations. First, trials that demonstrate PTP sustain a minimum level of potentiation during the trains that is greater than trials that do not demonstrate PTP. Second, the maximum level of potentiation during the trains does not differentiate PTP and non-PTP

trials below 50 Hz. Third, a number of trials reach maximum levels of potentiation during individual trains that surpass the minimum levels predicted for PTP induction, however, this potentiation is not sustained between the trains and these trials do not demonstrate PTP. In addition to these observations, we have shown that the level of sustained potentiation during train stimulation is correlated with the presence or absence of PTP.

Our results suggest that the minimum level of potentiation is sustained between the trains spaced 1 s apart. Only F_2 , with a decay constant of nearly 1 s, can carry the "memory" of one train to the next. However, we cannot be sure of a direct dependence of PTP on the level of potentiation due to F_2 . Consequently, for modeling purposes we have defined another term, S , with the dynamics and time constant similar to F_2 . We hypothesize that either F_2 is identical to S , or that both terms are initiated by a common underlying process. In this way, S is able to act as an indicator of MSP during transient potentiation and drive the induction of PTP in our model.

Modeling short term plasticity in the ELL

In this study, we have taken a novel approach to describe the five processes (F_1, F_2 , PTP, D_1 and D_2) underlying the dynamics of short-term plasticity at the StF-PC synapse. F_1 , F_2 , D_1 and D_2 have been modeled as exponentially decaying *FD*-type processes (Varela et al. 1997). The more complex dynamics of PTP were modeled by an enzymatic network interaction between an autoactivatable kinase, such as $CAMKII\alpha$, and a phosphatase. Most importantly, the threshold PTP induction was linked to MSP during train stimulation, through S , an F_2 -like process.

The completed model exhibits both the modest potentiation seen during the 1 Hz trains, and the frequency dependent transient potentiation observed at 5 - 100 Hz. The model also shows the observed threshold between 1 and 5 Hz for PTP induction, and the frequency independent amplitudes of PTP demonstrated above 5 Hz. This novel combination of modeling approaches has enabled us to describe the synaptic dynamics of the StF-PC synapse and provide a quantitative description of the relative contribution each synaptic process at various stimulation frequencies. These constraints will be useful in determining the underlying causes of plasticity and enable the investigation of potential functions of synaptic plasticity in network models involving closed loop feedback to the ELL.

Functional Significance of Short Term Synaptic Plasticity in ELL Processing

It has been proposed that the direct feedback pathway acts as a sensory searchlight that enhances the detection of weak signals. The differences in amplitude and duration of potentiation attained during transient potentiation versus PTP suggest that these forms of plasticity may have different roles in this type of ELL processing.

A closely related species of knife-fish, *Apteronotus albifrons*, is able to capture prey in less than 1 s after detection (Nelson and MacIver 1999). Depending on the speed the fish is swimming, the transdermal voltage change across an individual electroreceptor lasts approximately 200 ms, corresponding to a change in peak firing rate of electroreceptor afferents of 1-2 spikes in 60 (Ratnam and Nelson 2000). However, the ability of the fish to detect and capture prey suggests that pyramidal cells must be able to encode such minute changes in firing rate.

It has been suggested that synaptic plasticity of the direct feedback pathway could function as part of a sensory searchlight that could enhance pyramidal cell function (Bratton and Bastian 1990). The searchlight hypothesis was first proposed by Crick, (Crick 1984), in relation to the visual system. This theory has been updated and described in reference to ELL (Berman, et al. 1997). One requirement of the theory is reciprocal excitatory connectivity. This is fulfilled by the reciprocal and topographic positive feedback loop between the ELL pyramidal cells and the Pd stellate cells. A second requirement, non-linearity in the responsiveness of the pyramidal cells, could be fulfilled by the voltage-dependence of pyramidal cell EPSPs which is likely due to the activation of persistent Na⁺ channels on pyramidal cell somata and dendrites (Berman et al. in press). This voltage dependent, non-linearity could result in a supralinear summation of EPSPs.

In their paper, Berman et al. (1997) hypothesized that “when electroreceptor and StF input arrive concurrently at a pyramidal cell, the StF input is enhanced greatly and therefore is very effective at bringing the cell above spike threshold.” In the current study, we have shown that trains of 50 Hz or greater delivered to the StF are able to transiently enhance pyramidal cell EPSP amplitudes up to three-fold in 100-200 ms. Furthermore, the most significant changes in EPSP amplitude occur within the first 20 to 100 ms of train stimulation. In the intact animal, the feedback through the StF has a delay of approximately 20-30 ms (J. Bastian, personal communication; Berman, et al. 1997). These time frames suggest that in addition to the voltage-dependence of EPSPs (Berman et al. 1997, 2001), transient potentiation (F₁ and F₂ facilitation specifically) of the direct feedback pathway could rapidly and dramatically potentiate the sensory

searchlight. The combination of synaptic enhancement and EPSP voltage-dependence might conservatively be expected to produce >400% increases in StF EPSP amplitudes; this would be expected to greatly enhance electroreceptor input as the fish advances towards its prey. The topographic nature of the StF feedback pathway implies that this enhancement is confined to pyramidal cells adjoining those activated by electroreceptor input; thus, the transient potentiation demonstrated in this paper is likely to contribute to the detection of the weak signals caused by prey objects.

Conversely, the duration of PTP extends longer than expected for prey capture. However, in a more complex environment, such as the tangled root masses of the flooded Amazon River basin (Crampton, 1998), a more persistent upregulation of the sensory searchlight may be necessary during scanning and foraging behavior (Lannoo, 1992).

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Conclusion

“Cracking” the Electrosensory Pyramidal Cell Code

The primary focus of this thesis has been to identify and characterize candidate codes utilized by electrosensory lateral line lobe (ELL) pyramidal cells. In the thesis introduction five questions were posed pertaining to the processing of sensory input. These will now be answered with respect to electrosensory processing by ELL pyramidal cells based on the results presented in Chapters 1-4 as well as the requirements for a neural code as outlined by Perkel and Bullock, 1968. The results of this thesis and the speculations about how pyramidal cell spike trains might be “decoded” by the torus (see *Discussion* sections of Chapters 1 and 3) are summarized in Figure 1. Aspects of this figure will be described throughout the conclusion.

Which features of stimulus are encoded?

The broadband stimulus (purple) injected intracellularly into ELL pyramidal cells (green) contains both low frequencies that correspond to prey (red) and higher frequencies that correspond to interactions with other fish (blue). In Chapters 1 and 3, ELL pyramidal cells are shown to extract stimulus upstrokes. Although this has been demonstrated previously (Gabbiani, 1996; Metzner, 1998), the stimuli used in these studies were generally low frequency amplitude modulations. The findings in the present work are novel because the stimuli contained a broader range of frequencies which enabled the separation of low and high frequency upstrokes as distinct *referents* represented in pyramidal cell spike trains. In addition, the intensity of low frequency stimulus upstrokes was identified as a possible third referent represented in pyramidal cell responses.

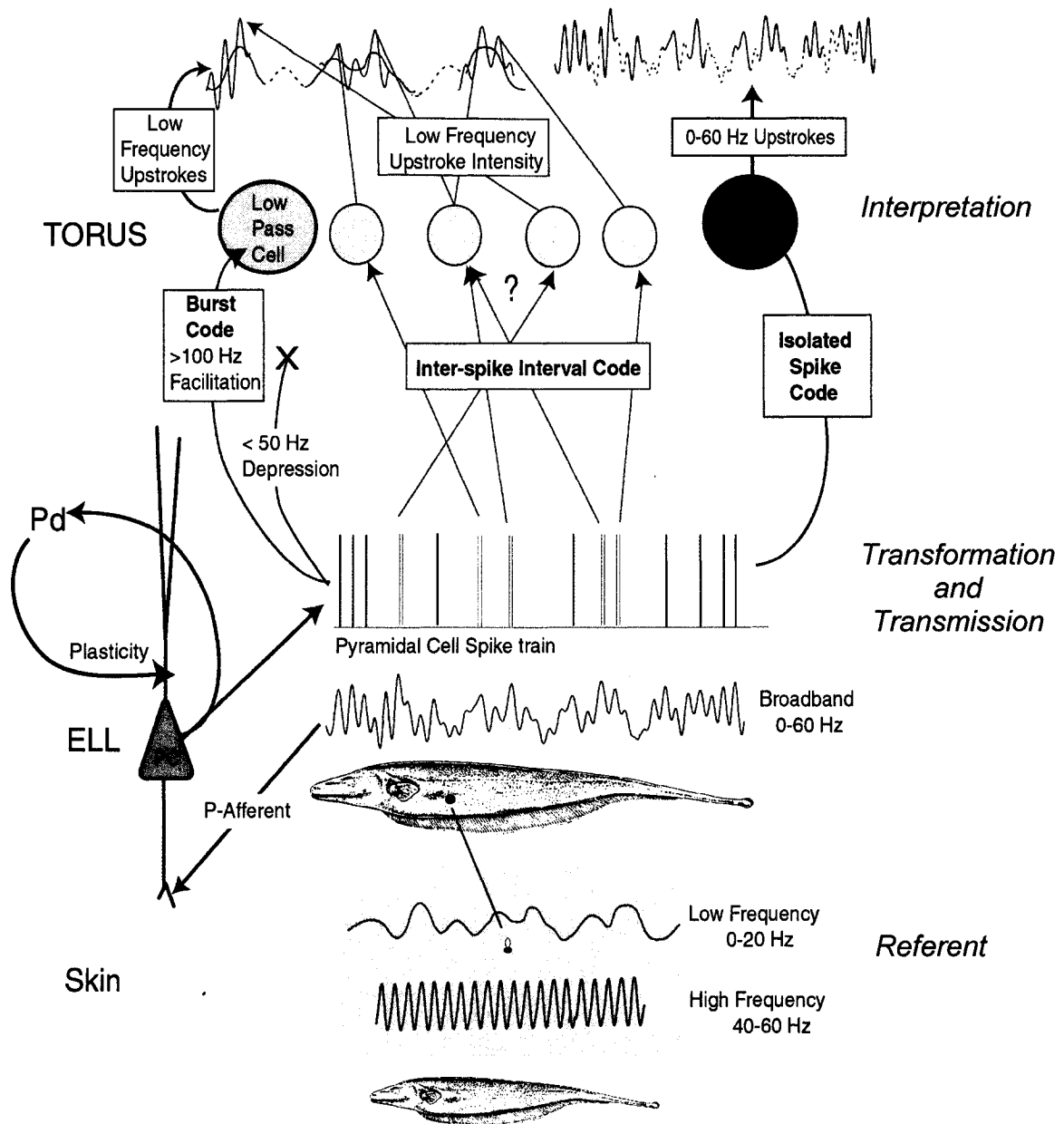


Figure 1: Thesis overview. Broadband stimuli (purple) consisting of low frequency prey-like inputs (red) and high frequency communication-like inputs (blue) were applied intracellularly to electrosensory pyramidal cells (green-PC). Spike train responses consisted of isolated spikes (blue) and bursts (red). These spike trains might be differentially decoded by low pass (orange) and broadband (purple) toral cells (Rose, 1993; Fortune, 2001). Feedback inputs from the nucleus praeminentialis (Pd) may modulate PC output.

The majority of neural responses analyzed in this thesis arise from the direct intracellular stimulation of ELL pyramidal cells *in vitro*. Thus, the neurons spike only in response to stimulus upstrokes that directly result in supra-threshold membrane depolarizations. This is true regardless of whether *in vitro* recordings are made from basilar (E-type) pyramidal cells or non-basilar (I-type) pyramidal cells. *In vivo*, I-type cells spike when they are relieved of intermediary granule cell inhibition (Saunders, 1984). Since granule cells receive electrosensory afferent input, inhibitory input would presumably increase with stimulus upstrokes and decrease with down strokes. Thus, if comparable stimulation were applied at the skin, it would be expected that I-type pyramidal cell responses would be correlated with the frequency and intensity of stimulus down strokes.

How are these features represented in the code?

In response to broadband stimulation, ELL pyramidal cell spike trains consist of both isolated spikes (blue) and bursts of spikes (red). The timing of burst events represents the *transformation* of the information pertaining to the occurrence of low frequency upstrokes (red) while the timing of isolated spikes represents the timing of upstrokes over a broad frequency range (purple) (Chapter 1). Although isolated spikes potentially code a broad frequency range, burst events are better indicators of low frequency events than isolated spikes (Chapter 1). Furthermore, the intensity of upstroke can be discriminated based on the burst inter-spike interval (ISI) (Chapter 3A).

The studies presented in Chapters 1 and 3 are not only novel with regard to the correlations between stimulus characteristics and spike train events, but also capitalize on the *in vitro* preparation and burst models to demonstrate a specific role for active

dendritic backpropagation in electrosensory processing. Burst firing is dependent on the active backpropagation of somatic spikes by the dendrite that results in a somatic depolarizing after potential (DAP). When backpropagation is prevented by the application of sodium channel blocker at the dendrite or by comparable modification in the Ghostbuster model, low frequency burst coherence and feature extraction by high frequency, burst-like events decrease (Chapter 1). Furthermore, the discrimination of stimulus intensity by burst ISIs can be influenced by changes in DAP intensity (Chapter 3B). Thus, the mechanism of transformation with respect to burst firing is dependent not only on the characteristics of the stimulus but also on the soma-dendritic interaction.

How precisely and reliably is the information coded?

In Chapters 2 and 3 it was shown that the timing of burst and isolated spike events, and the duration of the doublet ISIs are reproducible over repeated stimulus presentations. These results indicate that pyramidal cells reliably encode information pertaining to the occurrence of stimulus upstrokes and their frequency and intensity characteristics. It was further demonstrated that there is minimal variability in the timing of isolated spike and burst events from trial to trial. With regard to burst events, this precision is of significant importance. The latency of the first spike of the burst with respect to the stimulus influences the probability that a burst event will occur on a given trial and thus affects the reliability of burst events (Chapter 2). In addition, the variability in the timing between the two doublet spikes sets the minimum time separation (ms) in order for the information carried by two different ISIs to be discriminated (Chapter 3).

Can the information be quantified?

The application of information theory in neural systems has provided a means for measuring information transfer (MacKay, 1952). The most commonly used measure of mutual information; the amount of information about the stimulus contained in spike train responses; estimates the lower bound of information transfer assuming a linear encoding scheme (Borst, 1999). However, the quantification of information in pyramidal cell spike trains by estimates of the lower bound or linear stimulus reconstruction, is relatively low in comparison to the information contained in electrosensory afferent input (Gabbiani, 1996; Metzner, 1998; Chacron, 2003). In some systems, estimates of the upper bound of mutual information suggest that linear encoding accounts for a large fraction but not all of the information in spike trains (Borst, 1999). One possibility is that electrosensory information is encoded non-linearly by ELL pyramidal cells (Gabbiani et al, 1996).

The feature extraction technique assumes that the neuron linearly sums incoming information then performs a threshold computation that results in a non-linear response, such as the exact timing of a spike or a burst (Gabbiani et al, 1996). This measure was used in Chapter 1 to quantify the discrimination of upstrokes by bursts versus isolated spikes. Bursts were shown to be better indicators of low frequency upstrokes. This method was expanded in Chapter 3 in order to ascertain the discrimination of stimulus intensity by burst ISIs and quantify the potential information contained in the interval code. The feature extraction and interval coding measures presented assume the target neuron is an ideal observer having no knowledge of the statistical properties of the stimulus or the probability of spike events. The target is capable of interpreting the information transferred in bursts, but there is no assumption as to how this is

accomplished. Thus, the interval coding measure developed in Chapter 3 is a measure of the potential information contained in pyramidal cell spike trains provided the receiving neuron can interpret 2-3 ms differences in ISIs. There is a significant increase in the information contained in the burst responses if ISIs are considered a neural code versus the information contained solely in the timing of the first burst spike.

How is the information in the pyramidal cell code interpreted?

The main hypothesis of this work was that burst firing is a salient feature in the coding of electrosensory stimuli. The results presented in this thesis demonstrate correlations between burst firing and stimulus characteristics and quantify the reliability, precision and the potential information transfer of the encoding scheme. While these results support the hypothesis, they do not prove that burst firing is part of the pyramidal cell code. The hypothesis is true if information in bursts is uniquely interpreted by the target neurons of ELL pyramidal cells.

Electrosensory pyramidal cell axons *transmit* information via the lemniscal pathway to cells in the torus semicircularis. Toral cells are tuned to the frequencies of electrosensory input at the skin (Rose, 1993; Fortune, 1997). Low pass toral cells (orange) respond to low frequency stimuli, while broadband cells (purple) respond to a range of frequencies. The synapses between the ELL and low pass cells depress in response to inputs < 50 Hz (blue arrow, X) but facilitate in response to very high frequency >100 Hz input (red arrow) (Fortune, 2001). These synaptic dynamics could suppress isolated spike responses and promote the transfer of information coded by bursts. Effectively, low frequency stimuli applied to the skin (red waveform) are coded by high frequency pyramidal cell burst firing (red spikes) which is selectively interpreted

by toral cell synaptic dynamics (red arrow) to recover timing of low frequency upstrokes contained in the stimulus (red upstrokes, upper most trace). Additional information about the intensity of low frequency upstrokes may be recovered if toral cells can discriminate differences in burst ISIs. There are many possible intrinsic, synaptic and network mechanisms by which the inter-spike interval code may be interpreted (Chapter 3A). In Figure 1, the inter-spike code (brown arrows) is interpreted (?-mechanism unknown) by a number of toral cells, each selective for a different ISI. It is also conceivable that a smaller number of toral cells might regulate their firing rates in a plasticity-dependent manner in response to differing ISIs (Phares, 2003). Presently, there is not enough known about the properties of the toral cells to narrow the possibilities.

It is apparent that the pyramidal cell code cannot be “cracked” or verified until it is known how toral cells interpret pyramidal cell responses. This information cannot be obtained from the ELL slice preparation. However, *in vivo* studies could be designed to provide insight into toral cell processing. For instance, the following experiment could assess whether or not the information contained in burst inputs is differentially transferred versus that provided by isolated spikes. First a pulse sequence is generated with pulse timing dictated by pyramidal cell spike trains obtained in response to known stimuli applied at the skin. Then a few fibers of the lemniscal pathway are stimulated using this sequence while recording from a receptive toral cell. Correlations between the toral cell membrane response and the pyramidal cell spike train as well as the original stimulus might provide insight into how pyramidal cell inputs are interpreted by toral cells. While this approach is somewhat crude, the stimulation protocol is similar to that used previously to study toral cell synaptic responses (Fortune, 2001). The difference is

that the stimulus is based on actual pyramidal cell inputs rather than tetanic stimuli. The toral cells would be responding to realistic temporal patterning of bursts and isolated spikes. This and other comparable studies may provide the evidence necessary to prove that pyramidal cell burst firing is a neural code.

Can information transfer be modulated?

Although the synaptic dynamics described in Chapter 4 were presented last, these experiments were chronologically done first. Chapter 4 dealt with plasticity of a direct feedback pathway to the ELL that terminated on the proximal apical dendrites of pyramidal cells. There are two points of interest with regard to the direct feedback pathway: (a) the cells that give rise to this pathway (Sas, 1983) are selectively responsive to low-frequency input and are hypothesized to signal the presence of moving objects (Bratton, 1990); (b) the direct feedback terminates on the dendritic region that actively back-propagates spikes and thus causes the somatic DAPs and burst discharge (Turner, 1994). However, at the time of this study, very little was known about pyramidal cell responses to naturalistic stimuli. Since the relevance of plasticity in the direct feedback pathway depends on how feedback influences pyramidal cell responses to natural stimuli, interpreting the results of this study was difficult. Looking back on these studies now, it is easier to see how plastic feedback from the nucleus praeminentialis (Pd) might regulate stimulus encoding by ELL pyramidal cells.

As discussed in Chapter 4, the direct feedback pathway has been hypothesized to be part of a sensory searchlight mechanism (Crick, 1984; Berman, 1997, 1999). The direct feedback pathway is a reciprocal and topographical loop between cells of the Pd and ELL pyramidal cells. Thus, as a fish moves by an object, pyramidal cells that are

stimulated early in the scan activate the feedback loop and influence the responses of those pyramidal cells stimulated later. The direct feedback pathway is predominantly excitatory (Chapter 4 and references therein) and so this feedback would be expected to enhance the pyramidal cell response to moving objects- hence the searchlight hypothesis. One requirement of the searchlight hypothesis is non-linearity in the pyramidal cell response that would transiently enhance activity. Active dendritic backpropagation is a non-linearity that plays a significant role in pyramidal cell processing by producing transient, high frequency burst responses. Although studies of the ELL pyramidal cell burst mechanism were being conducted at the time, this obvious non-linearity was not included in the discussion of Chapter 4. Feedback inputs from Pd enter the ELL as the stratum fibrosum (StF) and make both excitatory and inhibitory synaptic contacts on the proximal apical dendrites and somata of pyramidal cells. The sodium channels responsible for active dendritic backpropagation and bursting are also located along the proximal apical dendrite. Thus, plasticity in the feedback inputs can influence pyramidal cell burst responses (Stuart, 2001) and backpropagating spikes can also influence the plasticity of feedback inputs (Gomez, 2005). The resulting pyramidal cell response depends on the stimulus, timing of the feedback inputs with respect to P-afferent input and the type of plasticity evoked at feedback synapses.

Studies of neocortical pyramidal cells have shown that synaptic input that is coincident with backpropagating action potentials can both increase the amplitude of dendritic spikes and induce bursting (Stuart, 2001). In the electrosensory system, direct StF stimulation has been shown to elicit bursts (Turner, 2002). Stimulation of the excitatory fibers of the StF evokes excitatory post synaptic potentials (EPSPs) that vary

in amplitude from 4-10 mV and have a significant and long lasting (> 50 ms) NMDA component of the EPSP (Berman, 1997, 2001). Presumably, plasticity at these synapses, which significantly increases EPSP amplitudes, could increase the level of depolarization in the dendrite, enhance DAP and promote burst responses. An interesting hypothesis is that facilitation and post-tetanic potentiation of the direct feedback pathway acts to enhance bursting and therefore optimize the detection of moving prey. Alternatively, the application of a GABA_A agonist at the dendrite has been shown to reduce the amplitude of the depolarizing after potential (DAP) and alter pyramidal cell firing rates (Mehaffey, submitted). The effects of changing DAP amplitude on burst coding were demonstrated using a simple model of the ELL burst mechanism in Chapter 3B. Thus, stimulation of inhibitory feedback fibers could decrease burst responses.

The fact that feedback inputs are localized to both promote and inhibit burst responses, suggests that direct feedback from the Pd might serve to gate burst firing and influence pyramidal cell coding (Krahe, 2004). This is comparable to the proposed role of cortical feedback in mediating the transition between burst and tonic firing in the thalamus (Sherman, 2002). Testing this hypothesis is difficult. Synaptic responses are transient and short-term synaptic plasticity decays continuously with time, but analysis measures such as coherence and feature extraction require pyramidal cell responses to be stationary for long periods of time. One possibility would be to induce potentiation in the excitatory pathway and then stimulate the StF at a fixed phase of low frequency, sine wave upstrokes. A comparable experiment could be designed to stimulate the inhibitory fibers of the StF. These studies may provide some insight into how excitatory and

inhibitory synapses might gate bursting and how the type and time course of plasticity might influence the encoding of low frequency stimuli with bursts.

The exact timing of backpropagating spikes with respect to synaptic input might also influence the type of synaptic plasticity manifested (Gomez, 2005). Spike timing dependent plasticity (STDP) has been demonstrated in mormyrid electric fish (Bell, 1997) and other systems (Bi, 1998, 2001). Although STDP has not yet been demonstrated in ELL pyramidal cells, the plasticity at StF excitatory synapses is anti-hebbian (Bastian, 1999). Pairing feedback input with post-synaptic depolarization results in synaptic depression, while pairing with hyperpolarization leads to potentiation. The role of the paired pre-synaptic and post-synaptic responses in pyramidal cell coding is unknown.

A significant advantage of the *in vitro* slice preparation is that results are interpreted with respect to single cell processing. The results of this thesis demonstrate the coding capacity of the single cell in the absence of feedback or network influences. *In vitro*, pyramidal cells code both the low and high frequency inputs simultaneously. However, *in vivo*, pyramidal cells switch between coding low and high frequencies depending on the spatial extent of the stimulus. In addition, pyramidal cell network oscillations in response to global stimuli are mediated by direct inhibitory feedback. A new avenue of experimentation might be to reintroduce feedback inputs to the slice preparation and attempt to elicit these *in vivo* pyramidal cell responses. These experiments could close the loop and provide valuable insight into how pyramidal cell coding may be dictated by feedback from the Pd.

Speculations

Recent *in vivo* studies have shown that when an electric fish is presented with a mixed stimulus consisting of a moving prey-like object in a background of > 30 Hz global input, toral cells are more direction sensitive than without the global input (Ramacharitar, 2004). This stimulus mimics the scenario of the fish hunting with other fish nearby. The results suggest that higher frequency stimuli enhance prey detection. Based on the results presented in Chapter 2 and 3, I speculate that the introduction of high frequency inputs promotes temporally precise burst firing and interval coding by pyramidal cells. First, high frequencies increase the slope of the upstroke which decreases the latency of the first spike and allowing time for the stimulus to influence the timing of the second burst spike. Second, high frequency stimuli introduce sharper down strokes that would limit the autocatalytic burst mechanism to a few spikes per upstroke. It would be interesting to know whether or not pyramidal cell burst responses were correlated with toral cell direction sensitivity. This would suggest a mechanistic role of pyramidal cell burst responses in sensory processing.

The role of burst firing in neural coding and sensory processing remains a hotly debated topic. These studies suggest that pyramidal cell bursts uniquely code specific features of electrosensory stimuli. In addition, these results provide insight into the role that intrinsic burst mechanisms and single cell responses may play in sensory processing and how these responses might be modulated by higher brain centers. Although these studies were conducted in the electrosensory system, the interpretation of the results may extend to other systems. Although the biophysical basis of thalamic bursting is entirely different from that of ELL pyramidal cells (Llinas, 1982), thalamic cell spike trains

consist of both isolated spike and burst discharge patterns (Sherman, 2001). Recent studies of thalamic cells have shown that burst responses are reliable, respond to distinct stimulus features, have specific frequency tuning properties, and thus, may play an important role in the encoding of natural visual scenes (Grubb, 2004; Lesica, 2004; Alitto, 2005). Furthermore, there is feedback from cortex to thalamus that can modulate thalamic responsiveness and perhaps bursting (Sherman, 2002). The concept of a “burst code” will likely remain in debate until evidence that burst responses are interpreted and uniquely transfer information between neural populations. The role of burst firing in sensory processing may only be finally elucidated in behavioural studies when burst firing is either compromised or enhanced and sensory processing altered in the predictable manner.

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