Predicting Cell-Type Specific Active Properties by Developing Multi-Compartment Models Using Databases and Electrophysiological Feature Constraints: Application to Interneuron Specific 3 (IS3) Cells in the Hippocampus

by

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A thesis submitted in conformity with the requirements for the degree of MSC-PHYSIOLOGY

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Abstract

In hippocampus, interneuron specific 3 (IS3) cells have been shown to make inhibitory synapses onto specific types of inhibitory interneuron dendrites with the ability to control their firing patterns. Morphological and synaptic aspects of IS3 cells are being examined, but what type, how much and where voltage-gated channels (VGCs) are present on IS3 cells has not been determined. Using a combination of the NEURON and MATLAB software environments, we have developed an approach that uses experimental IS3 data to generate databases of multi-compartment models that show appropriate activity for IS3 cells. Given their correspondence with data, our models predict relative conductance balances and distributions of different channel types in IS3 cells and can be used as a basis for understanding the functional roles of IS3 cells in hippocampus, a central structure in memory formation.
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1 Introduction

1.1 The Hippocampus

1.1.1 Structure

In Neurophysiology, the hippocampus is a commonly studied brain structure. Anatomically (Witter, 2010; Amaral and Lavenex, 2006), the hippocampal formation is divided into the dentate gyrus, the hippocampus proper and the subiculum. The hippocampus proper is further divided into the Cornu Ammonis (CA) areas, comprising of CA1, CA2 and CA3. Collectively, the excitatory pathway from dentate gyrus to CA3 and then to CA1 is known as the trisynaptic loop. Classically, this hippocampal pathway has been viewed as unidirectional but more recent findings have shown that this is not always the case since there are also serial and parallel pathways (Amaral and Lavenex, 2006) as well as inhibitory pathways going in the opposite direction (Jackson et al, 2014). These areas are further subdivided into layers where the different morphological compartments of different cell types are found. In CA1 these layers are: stratum oriens/alveus (SO/A), stratum pyramidale (SP), stratum radiatum (SR) and stratum lacunosum-moleculare (SLM).

1.1.2 Function

Functionally, the hippocampus is associated with a number of learning and memory tasks such as pattern separation through dentate gyrus sparsity (Piatti et al, 2013), spatial navigation through CA3 and CA1 pyramidal cell firing dynamics (O'Keefe and Dostrovsky, 1971) and long term potentiation of synapses in the CA1 during learning tasks (Whitlock et al, 2006). In other words, the hippocampus is a functionally rich and central structure in the formation of new memories. In fact, damage to the human hippocampus has often been associated with anterograde amnesia, such as during Alzheimer’s disease and other aging-related dementias (Babiloni et al, 2009). Additionally, networks found in the hippocampus are known to generate neuronal population rhythms such as the theta rhythm (3-12 Hz; Buzsaki, 2002), which has been associated with a number of the learning and memory tasks listed previously. In particular, theta rhythms have been associated with spatial navigation as well as memory encoding and retrieval (O’Keefe and...
Reece, 1993; Siegle and Wilson, 2014). Networks in hippocampus have also been shown to
generate fast oscillatory states (130-230 Hz), or, sharp wave associated ripples (SWR). SWRs
have primarily been associated with memory consolidation (Girardeau et al, 2009). As well,
hippocampal networks can also generate 25-55 and 60-100 Hz rhythms that are respectively
known as slow and fast gamma rhythms (reviewed in Colgin, 2015). Functionally, slow and fast
gamma rhythms occur during slow and fast running velocities, respectively (Zheng et al, 2015).
While slow gamma is known to occur during memory retrieval processes and is driven by input
from CA3, fast gamma occurs when new memories are being encoded and is driven by input
from medial entorhinal cortex (Bieri et al, 2014; Zheng et al, 2015). Also, gamma rhythms are
often nested in theta rhythms and are thought to be associated with working memory (Lisman,
2010; Colgin, 2015). Furthermore, dysfunction of cellular and network activity in the
hippocampus has also been known to lead to pathological conditions such as epilepsy (Jefferys,
2010).

1.2 Hippocampal Inhibitory Interneurons

1.2.1 Interneuron Diversity and Roles

The hippocampus contains a large diversity of local inhibitory interneurons that possess
differences in morphology, electrophysiology and molecular markers (Klausberger and Somogyi,
2008; McBain and Fisahn, 2001). These differences allow for GABAergic connections to
particular morphological sites on pyramidal cells (e.g. somatic or dendritic), which provide a
diversity of cell-type-specific inhibitory control over information integration as well as firing
dynamics. Inhibitory interneurons represent roughly 10-15% of the total amount of neurons in
the cortex (Vida, 2010) yet exert particular control over network dynamics and behavioral states
(Kepecs and Fishell, 2014). Oriens-Lacunosum Moleculare (OLM) interneurons in particular are
thought to intrinsically (i.e. through spike refractoriness and the presence of hyperpolarization-
activated current) contribute to population theta rhythms through feedback inhibition of
hippocampal pyramidal cells (Maccaferri and McBain, 1996; Kispersky et al, 2012).
Collectively, local inhibitory input onto pyramidal cells in the hippocampus spans across most of
the morphology of pyramidal cells, exhibiting cell-type specific differences in timing and
magnitude of inhibitory control both in vitro and during different behaviors (Klausberger and
Somogyi, 2008; Katona et al, 2014; Varga et al, 2012; Lapray et al, 2012). For example,
axoaxonic cells target axon initial segments, PV+ basket cells target soma and proximal dendrites, bistratified and ivy cells target apical and basal dendrites and OLM cells target distal apical dendritic tufts (Klausberger and Somogyi, 2008). Although it is clear that hippocampal inhibitory interneurons exert control over pyramidal cells and synchronize local network activity, it is less clear how the activities of these interneurons are controlled themselves.

### 1.2.2 Interneurons that Inhibit Other Interneurons

In addition to inhibitory control over pyramidal cells, there is quite a bit of literature describing various mechanisms of inhibitory control over inhibitory interneurons themselves. Because these mechanisms involve inhibiting inhibitory cells, they can also be seen as circuitries that permit the disinhibition and increase of gain in pyramidal cells. For example, disinhibition can occur through self-inhibiting autapses, local inhibitory connections between interneurons that mainly target pyramidal cells and input from long-range inhibitory projections, such as from the medial septum or the medial entorhinal cortex (Chamberland and Topolnik, 2012). Particularly, disinhibition can also occur when inhibition originates from a class of inhibitory interneurons that exclusively target other interneurons (Acsády et al, 1996b; Gulyás et al, 1996). In other words, contrary to other interneurons these types of cells mainly target inhibitory interneurons and not pyramidal cells.

Initially, this group of cells was identified based on evidence that rat hippocampal interneurons expressing calretinin (CR) or vasoactive intestinal polypeptide (VIP) synapse selectively onto other interneurons (Gulyás et al, 1996). Importantly, there has been confirmation that these cell types can be found in the human hippocampus (Urbán et al, 2002) as well as cortical regions outside of the hippocampus (Caputi et al, 2009). For example, disinhibitory VIP+ cells in auditory and medial prefrontal cortices of mice have been shown to inhibit somatostatin (SOM)- and parvalbumin (PV)-expressing interneurons, which, respectively, control the input and output of pyramidal cells (Pi et al, 2013). In this same study, these cortical VIP+ interneurons showed an increase in activity following reinforcement feedback in an auditory go/no-go task. Similar findings are seen in the primary somatosensory barrel cortex where pyramidal cells from the primary vibrissal motor cortex primarily activate VIP+ interneurons, which primarily inhibit SOM+ interneurons (Lee et al, 2013). These somatosensory VIP+ interneurons show increased activation during whisking behaviors in mice, while SOM+ interneurons show decreased
activation. Furthermore, in the mouse visual cortex, VIP+ interneurons also preferentially inhibit SOM+ interneurons (Pfeffer et al., 2013). In fact, in vivo calcium imaging of cells in mouse visual cortex reveals that VIP+ interneuron activity increases during locomotion and this increase is modulated by the presence of visual stimulation. Accordingly, SOM+ interneurons in visual cortex demonstrate decreased activation during locomotion (Fu et al., 2014). This disinhibitory circuit in the visual cortex seems mainly to be important for regulating the enhanced neural activity and adult cortical plasticity that occurs during locomotion (Fu et al., 2015). Interestingly, VIP+ interneuron activity also increases during locomotion when examined in the barrel and auditory cortices (Fu et al., 2014).

In terms of hippocampal disinhibitory cells, or interneuron-specific (IS) interneurons, these cells are divided into three different types, based on neurochemical and anatomical differences (Acsády et al., 1996a). The first type, IS type 1 (IS1) cells, expresses CR and mainly targets calbindin (CB)- and CR- expressing interneurons and appears to form characteristic dendro-dendritic gap junctions with different cells (Acsády et al., 1996a; Chamberland and Topolnik, 2012; Gulyás et al., 1996). The second type, IS type 2 (IS2) cells, mainly targets CB- or VIP-expressing interneurons as well as cholecystokinin (CCK)/VIP expressing basket cells (Acsády et al., 1996a). IS2 cells are also known to express VIP but not CR (Francavilla et al., 2015). The third type is the IS type 3 (IS3) cell.

1.3 Interneuron Specific 3 Interneurons in Hippocampus

1.3.1 Identification

Anatomically, IS3 cell somas are located in the SR layer along the SP border with axons projecting into the SO/A and dendrites projecting into the SLM where they preferentially synapse onto type 1a metabotropic glutamate receptor (mGluR1a) expressing OLM cells (Acsády et al., 1996b). In terms of neurochemical markers, IS3 cells are known to co-express both VIP and CR and might also express enkephalins (Francavilla et al., 2015; Blasco-Ibáñez et al., 1998).

1.3.2 IS3 Cell Control over OLM Cells

Through photostimulation experiments, it has been shown that IS3 cells provide a major local source of inhibition to OLM cells (Chamberland et al., 2010). Interestingly, in these same
experiments, IS3 cells were not found to exhibit long-term potentiation (LTP), whereas long-range septohippocampal inhibitory projections did show LTP as well as more persistent and higher amplitude inhibition than what was exhibited by IS3 cells. Already, this seems to suggest different types of control over information processing through dishinhibition. More recently, IS3 cells have been characterized and they have been shown to primarily synapse onto other interneuron dendrites with the ability to control their firing patterns (Tyan et al., 2014). More specifically, it was shown that IS3 to OLM synapses have weak transmission efficacies (i.e. low release probability, 5.9 pA quantal size and ~2-3 release sites), relative to other cell types. It was also shown that high frequency single cell IS3 firing (i.e. around 100 Hz) is necessary to elicit synaptic summation in OLM cells. These results seem to suggest that single IS3 spikes are not sufficient in eliciting large enough inhibitory currents to control OLM cell firing. However, this study also showed that IS3 cells could control OLM cells (i.e. through post-inhibitory rebound spiking) when many IS3 cells were optogenetically activated synchronously. Interestingly, this control was optimal when IS3 cells were optogenetically stimulated in theta rhythms. As it happens, OLM cells are known to phase lock well with theta rhythms (Kispersky et al., 2012). For these reasons, we assume that IS3 cells exhibit control over OLM cells by having multiple IS3 cells fire synchronously. How this could happen is if IS3 cells are able to fire with minimal inputs, so that multiple IS3 cells with closely timed spikes (i.e. less than 10ms interspike intervals so that synaptic summation can occur) could emerge more readily for a given input. In this way, IS3 cells would be able to exhibit control over OLM cells. As it stands, it is known that IS3 cells are highly excitable with a high input resistance (400-600 Mohms) and a small rheobase (30-50 pA) (Tyan et al., 2014). Additionally, IS3 cells have also been found to target other interneuron classes of O/A interneurons, including basket, bistratified and oriens-oriens (i.e. interneurons whose whole morphology is situated within the oriens layer) cells (Tyan et al., 2014).

1.3.3 Potential Roles

As it stands, it is not known what inputs may be synapsing onto IS3 cells. Based on anatomical evidence, IS3 cells likely receive excitatory inputs from the entorhinal cortex via the perforant path, from the CA3 area via Schaffer collaterals or from CA1 local collaterals (Francavilla et al., 2015). This suggests potential roles for IS3 cells during encoding (entorhinal cortex) and retrieval tasks (CA3). Additionally, IS3 cells may be controlled by inhibitory inputs from local
CR+/VIP+ targeting IS1 cells in hippocampus (Gulyás et al., 1996), which may contribute to the state-dependent activation of IS3 cells by balancing the excitation with inhibition during particular behavioral/network states (Francavilla et al., 2015).

Also, in vivo recordings from hippocampal OLM cells in rats have shown that OLM cells typically do not spike during SWRs and, on average, even show decreased activation (Katona et al., 2014). Bistratified and PV+ basket cells, on the other hand, show increased activation during SWR (Katona et al., 2014). Therefore, during SWRs it may be that excitatory CA3 Schaffer collateral input drives both bistratified cell activation as well as IS3 cell inhibitory inputs to OLM cells. Essentially, this would serve to amplify pyramidal cell excitatory inputs onto distal apical dendrites (i.e. from entorhinal cortex) while dampening excitatory inputs onto proximal dendrites (i.e. from CA3). On the other hand, these findings could also be explained by long-range GABAergic septal inputs inhibiting OLM cells during SWR (Chamberland et al., 2010; Katona et al., 2014).

### 1.4 Voltage-Gated Channels

Although it is important to clarify the role(s) of IS3 cells within the hippocampal microcircuit, nothing is currently known about the voltage-gated channels (VGC) found in IS3 cells. The VGC types, kinetics, conductance balances and morphological distributions play a large role in controlling the electrophysiological features that are seen in any specific cell type (Hille, 2001). VGCs are voltage-sensitive transmembrane proteins that control the influx and outflow of ions during events such as action potentials. To do this, VGCs act as transmembrane pores that open and close in response to changes in membrane potential. In open states, VGCs allow the passage of ions across the lipid membrane of the cell. This essentially contributes to the ion gradient between the intracellular and extracellular spaces and helps shape the electrophysiological features of neurons. The gating kinetics of VGCs are mostly determined by their genetically encoded subunit proteins. Although there are many types of VGCs, here we will only discuss sodium and potassium VGCs. This is because nothing is currently known about IS3 cell VGC properties, and sodium and potassium channel types are a reasonable initial focus due to their roles in spiking mechanisms.
1.4.1 Sodium Channels

Generally, sodium channels are composed of one alpha subunit with two accessory beta subunits (reviewed in Catterwall, 1992). In mammals, there are eleven genes (SCN1A-SCN11A) that encode sodium channel alpha subunits, of which only nine are functional (Na\textsubscript{v}1.1-Na\textsubscript{v}1.9). In the central nervous system (CNS) it is mostly four alpha subunit isoforms (Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.3 and Na\textsubscript{v}1.6) that are highly expressed, which we will focus on here (reviewed in Goldin, 2001).

Each alpha subunit is composed of four repeated domains (I-IV), each possessing six transmembrane segments (S1-S6). Region S4 acts as the voltage sensor for sodium channels, mainly due to its positive charge, which causes it to move towards the extracellular space during changes in voltage. This makes the channel more permeable (specifically the regions between S5 and S6) to sodium ions, allowing an influx of Na\textsuperscript{+} into the intracellular space (Catterwall, 1992). Additionally the region linking domains III and IV functions as an inactivation gate that reduces ion permeability during prolonged activation (Catterwall, 1992).

As well, there are three genes (SCN1B-SCN3B) that encode sodium channel accessory beta subunits (Na\textsubscript{v}B1.1-Na\textsubscript{v}B3.1). All three of these genes are expressed in CNS and modulate the kinetic properties of sodium channels (Catterwall, 1992; Goldin, 2001).

In terms of the different types of sodium channels, these are mainly divided based on whether they are transient or persistent (i.e. whether or not they inactivate, respectively). The degree of sodium channel persistence is largely determined by alpha and beta subunit composition. For example, it has been shown that channels with Na\textsubscript{v}1.1 subunits demonstrate large persistent currents at negative membrane potentials that decrease with increasing membrane potential. On the other hand, Na\textsubscript{v}1.6 subunits also generate persistent currents that increase with increasing membrane potential. Lastly, Na\textsubscript{v}1.2 seems to generate the least amount of persistent currents of the alpha subunits (Smith et al, 1998). In terms of the beta subunits, co-expression of any beta subunit with alpha subunit Na\textsubscript{v}1.2 generates sodium currents with shifted activation and inactivation curves towards higher membrane potentials. In terms of persistence, co-expression of beta subunit Na\textsubscript{v}B1.1, beta subunit Na\textsubscript{v}B2.1 and alpha subunit Na\textsubscript{v}1.2 show no increase in persistent current. On the other hand, co-expression of beta subunit Na\textsubscript{v}B3.1 with alpha subunit Na\textsubscript{v}1.2 shows a significant increase in persistent current. Furthermore, co-expression of beta subunit Na\textsubscript{v}B3.1 and beta subunit Na\textsubscript{v}B2.1 with alpha subunit Na\textsubscript{v}1.2 shows an even larger
increase in persistent current (Qu et al., 2001). In other words, both alpha and beta subunits play key roles in determining the degree of persistence of sodium channels.

1.4.2 Potassium Channels

Relative to sodium channels, potassium channels are more complex, mainly due to the larger number of genes that encode different potassium subunits. Potassium channels are divided into three categories: voltage-gated K\(^+\) channels and Ca\(^{2+}\)-activated K\(^+\) channels, leak K\(^+\) channels and inward rectifier K\(^+\) channels. Like sodium channels, potassium channels are comprised of both pore-forming alpha subunits and auxiliary beta subunits that interact with the main channel complexes (reviewed in Coetzee et al., 1999). Here we will focus on the alpha subunits that comprise voltage-gated K\(^+\) channels (i.e. Kv1 to Kv9 subunit subfamilies). To begin, voltage-gated K\(^+\) channels are assembled using a combination of four alpha subunits in either homomultimeric or sometimes heteromultimeric structures. Additionally, each subunit contains six transmembrane helices (S1-S6) that are assembled around a potassium selective pore (Yellen, 2002). Similar to sodium channels, the S4 transmembrane segment acts as the major voltage-sensing element in the structure (Larsson et al., 1996). We will focus here on three types of voltage-gated potassium channels: slowly activating and deactivating delayed rectifier potassium channels, fast activating and deactivating delayed rectifier potassium channels and rapidly inactivating potassium channels. The kinetics found in each of these types of voltage-gated potassium channels is largely determined by their constituent alpha subunits and is modulated by auxiliary beta subunits.

For example, slowly activating and deactivating delayed rectifier potassium current can be generated from channels composed of Kv2.1 and/or Kv2.2 subunits, likely in combination with Kv5-9 subunits (i.e. slow delayed rectifier potassium channels; Lien et al., 2002; Coetzee et al., 1999). Fast activating and deactivating delayed rectifier potassium current can be generated from channels composed of Kv3.1 and/or Kv3.2 subunits (i.e. fast delayed rectifier potassium channels; Lien et al., 2002). Additionally, Kv3.1 alpha subunit composition seems to yield faster deactivation time constants than channels with Kv3.2 alpha subunit composition (Hernández-Pineda et al., 1999). Lastly, rapidly inactivating potassium current, or A-type potassium channels, can be generated from channels composed of either Kv3.3 (Fernandez et al., 2003), Kv1.4, Kv3.4, Kv4.1, Kv4.2 or Kv4.3 alpha subunits (reviewed in Cai et al., 2007 and Carrasquillo and
Nerbonne, 2014). Subunit expression of A-type potassium channels also seems to be region specific, such as in the hippocampus where Kv4.2 is highly expressed (Carrasquillo and Nerbonne, 2014), or in the hippocampal CA1 SO/A layer where Kv4.3 is highly expressed (Lien et al, 2002).

1.5 Electrophysiological Features

With different balances and distributions of the VGCs mentioned in the previous sections, it is possible to elicit a variety of different electrophysiological regimes (e.g. irregular spiking, spike frequency adaptation, high frequency spiking, depolarization block). This is possible because VGCs have direct effects on electrophysiological dynamics such as how spikes are initiated, cell excitability and action potential shape and propagation. Importantly, the effects of VGCs are also often dependent on the morphology and passive properties of the cell. IS3 cells in particular elicit several different spiking regimes such as irregular firing when held close to threshold (Tyan et al, 2014) and depolarization block at current injections greater than ~250pA. Here we will discuss some of potential mechanisms through which these features can occur.

1.5.1 Irregular Firing

Because IS3 cells can exhibit irregular firing when close to threshold we assume that it is important biologically since irregularly firing neurons have been recorded in vivo in cortex (Noda and Adey, 1970; Softky and Koch, 1993; Holt et al, 1996). Though there are several possible measures for irregular firing in neurons (Softky and Koch, 1993; Holt et al, 1996), here we use the coefficient of variation of the interspike interval (\(ISI\ CV = ISI\ Standard\ Deviation/ISI\ Mean\)). Importantly, neurons with ISI CVs greater than 1 are considered irregular firing whereas neurons with ISI CVs less than 0.5 are considered regular firing (Stiefel et al, 2013).

Early studies of irregular firing in awake and behaving macaque monkeys have shown that nonbursting cells firing at greater than 300 Hz in primary visual and extrastriate cortices, show high spike time variability (i.e. using the ISI CV as a measure) as well as high spike count variability (i.e. using the ratio of the variance to the mean for the number of spikes evoked by a constant stimulus as a measure) (Softky and Koch, 1993). In this case, multi-compartment models only captured irregular firing when fast and strong nonlinear dendritic channel densities were included (i.e. with particularly large delayed rectifier potassium currents). Another study
showed that recordings from cat neocortical slice neurons \textit{in vitro} were regular firing, whereas recordings from primary visual cortex neurons \textit{in vivo} were irregular (i.e. using the relative difference between adjacent interspike intervals as a measure) during both visual stimulation and current injections (Holt \textit{et al}, 1996). In this case, integrate-and-fire models only captured irregular firing when receiving large amounts of background synaptic activity. Interestingly, although both visual stimulation and current injections evoked irregular firing \textit{in vivo}, spike times were more irregular during visual stimulation. This suggests that dendritic nonlinearities (i.e. which are bypassed during somatic current injections) may be amplifying irregular firing. An additional study has also shown that irregular firing could be elicited in rat prefrontal cortical slices \textit{in vitro} by using dynamic clamp to inject simulated stochastic background synaptic activity in pyramidal cells (Destexhe \textit{et al}, 2001). In general, between the large amounts of synaptic activation received and active dendritic properties, neurons \textit{in vivo} can often be found in noisy high-conductance states that appear to be stochastic, which promotes irregular firing (as reviewed in Destexhe \textit{et al}, 2003).

In terms of intrinsic mechanisms, it has also been shown that irregular firing in mouse cortical interneurons \textit{in vitro} can be generated through fast activation of potassium time constants and the introduction of Gaussian white noise to simulate stochastic gating (Stiefel \textit{et al}, 2013). Using Hodgkin-Huxley type models, these faster potassium activation time constants were also shown to increase the bistable region in which irregular spiking can occur. Interestingly, fast activation time constants played a larger role in modulating ISI CVs than noise amplitude, regardless of the type of noise used (i.e. Gaussian white noise, power-law noise and Ornstein-Uhlenbeck noise were all analyzed). Importantly, Gaussian white noise is a simple intrinsic noise model relative to more detailed noise models where noise can be generated from simulations of realistic stochastically gating channel models (Fox, 1997; Dorval, 2006; Goldwyn \textit{et al}, 2011). On the other hand, Gaussian white noise is known to be sufficient for modeling stochastic gating in order to generate irregular firing (based on Stiefel \textit{et al}, 2013) and other subthreshold membrane potential properties (e.g. Morin \textit{et al}, 2010; Sritharan and Skinner, 2012). Also, since the actual biological details of stochastic channel gating in IS3 cells remain unknown, it seems reasonable to use Gaussian white noise as a simple model of stochastic gating.

Furthermore, previous work has also shown that stochastic gating can often have a generally detrimental effect on spike time precision (Schneidman \textit{et al}, 1998; Schreiber \textit{et al}, 2004; Ozer \textit{et
al, 2009), which, considering the weak synaptic transmission properties of IS3 cells to OLM cells could easily reduce IS3 control over OLM cells. However it has also been shown that the presence of subthreshold membrane potential oscillations can have the effect of stabilizing spike time precision by minimizing membrane potential variance while at the trough of membrane potential oscillations (Schaefer et al, 2006). Additionally, this effect seemed to occur robustly across multiple cell types. Importantly, there are several ways in which subthreshold membrane potential oscillations can occur and, often, the inclusion of a stochastically gating noise mechanism is necessary for this to occur (Schneidman et al, 1998; Schreiber et al, 2004; Morin et al, 2010; Sritharan and Skinner, 2012; Yoshida et al, 2011). In this sense, although the inclusion of a stochastic gating mechanism should intuitively decrease spike time precision, by including other components that are known to allow subthreshold membrane potential oscillations to occur, spike time precision (i.e. upon activation) may, theoretically, not be too negatively affected.

1.5.2 Depolarization Block

Another electrophysiological regime of interest is depolarization block. During depolarization block, it is observed that with sustained current injections of increasing strengths, neurons will eventually stop firing (Bianchi et al, 2012). Because of the large magnitude of current injections that are necessary for depolarization block to occur it has often been assumed that depolarization block is not physiologically relevant. However, by considering the amount of excitatory synaptic input that neurons can receive, it becomes evident that neurons are in fact capable of reaching depolarization block in a network context (Bianchi et al, 2012). In fact, depolarization block seems to occur in interneurons during epileptic events leading to a reduction of inhibition in hippocampal (Ziburkus et al, 2006; Karlócai et al, 2014; Yi et al, 2015; Sanjay et al, 2015) and cortical (Cammarota et al, 2013) networks. Several mechanisms through which depolarization block occurs have been proposed. In a CA1 pyramidal cell model it was found that the half-activation voltage of the delayed rectifier potassium needed to be shifted to the right relative to the half-activation voltage of transient sodium, and that there needed to be a small transient sodium window current (i.e. where the activation and inactivation steady-state curve overlap; Bianchi et al, 2012). As well, this suggested that any additional inward current, such as from persistent sodium, would facilitate depolarization block. In another study (Tucker et al, 2012), depolarization block was investigated in midbrain substantia nigra dopamine neurons using
dynamic clamp techniques to vary the distribution, density and gating of transient sodium channels. In this case, decreased somatic sodium led to a decrease in spike frequency as well as an increase in susceptibility to depolarization block, and vice versa. As well, when sodium channels were restricted to the soma, there was an abnormally high firing rate because of excessive local subthreshold sodium currents.

1.6 Interpretation of Dendritic Calcium Recordings

One method of predicting potential distributions of VGCs along the dendrites of neurons is to look at dendritic calcium imaging recordings after eliciting somatic action potentials. Since these somatic spikes are expected to backpropagate into the dendrites, this is typically reflected by an increase in the calcium signal at various points along the dendrites. Calcium signals at large distances from the soma, for example, would therefore imply that VGCs must be present at these distances in order to propagate the action potential and activate calcium channels. Furthermore, the calcium signal could be deteriorating due to either a reduction in calcium channels past a certain distance along the dendrites, or because of a reduction in other VGCs that are necessary for action potentials to propagate. In other words, calcium imaging provides qualitative evidence that dendritic VGCs are present but does not provide quantitative evidence regarding channel types and distributions that exist in IS3 cells.

1.6.1 Calcium Imaging in Pyramidal Cell Dendrites

Despite the limitations of dendritic calcium imaging, previous studies of hippocampal CA1 neurons have shown that calcium signals often predict the presence or absence of backpropagating action potentials quite well. For example, simultaneous dendritic patch-pipette and calcium signal recordings have demonstrated that action potential amplitude decays minimally with dendritic distance in CA1 pyramidal cells (Spruston et al, 1995). These single backpropagating spikes were also accompanied with non-decaying elevations in dendritic calcium signals up until 400 µm, and the application of tetrodotoxin (TTX), a sodium channel blocker, significantly increased the decay of action potentials with distance from soma. Additionally, during somatic spike trains, both dendritic spikes and calcium signals seemed to decrease in amplitude with distance from soma (Spruston et al, 1995). A further study of CA1 pyramidal cells (figure 1, left; Golding et al, 2001) showed that they could be subdivided into strong propagating neurons (i.e. little spike amplitude deterioration along ~400 µm of dendrites)
and weak propagating neurons (i.e. possess a sharp decline in spike amplitude past 300 µm along the dendrites). Similarly, strong propagating pyramidal cells also exhibited non-decaying calcium signals up until 400 µm whereas weak propagating neurons exhibited sharp declines in the calcium signal at distances past 300 µm along the dendrites. Furthermore, the difference between strong propagating and weak propagating was not dependent on morphological properties. In fact, modeling work showed that the difference could be explained by having larger sodium channel density increases with distance from soma and smaller delayed rectifier potassium channel density increases. These changes in channel densities could also account for differences in action potential shape (i.e. halfwidths, and rise times) between the two groups (Golding et al., 2001). It has been shown that sodium channel densities are more or less uniform across the somatodendritic axis of pyramidal cells (Magee and Johnston, 1995). On the other hand, calcium channel distributions in pyramidal cell dendrites are more complex, with L-, N-, P-, Q-, R- and T- type channels mainly contributing to spike-triggered calcium entry into the soma and first 50 µm of proximal dendrites and mainly R- and T- type channels contributing at distances greater than 100 µm along the dendrites (Christie et al., 1995). With these findings in mind, mechanisms of action potential backpropagation in CA1 pyramidal cells are likely due to a combination of dendritic channel distributions. In general, calcium signal decay along the dendrites appears to be accurately predictive of action potential amplitude decay in these cells.

1.6.2 Calcium Imaging in OLM Cell Dendrites

Regarding inhibitory interneurons in the hippocampal CA1 region, several cases have been explored. Patch-clamp recordings from SO/A interneurons (i.e. putative OLM cells) showed that high densities of dendritic sodium and potassium channels allow for constant amplitude, high velocity spikes along dendritic distances as far as 120 µm (Martina et al., 2000). As would be expected, application of TTX caused spike amplitudes along the dendrites to decrease. Likewise, another study showed that there is only a small decline in the calcium signal along the dendrites of OLM cells (Topolnik et al., 2009). More specifically, a strong calcium signal was seen at distances as far as 140 µm from the soma, which seems to suggest that the calcium signal in OLM cell dendrites, like in CA1 pyramidal cells, is predictive of backpropagating action potential amplitude (figure 1, middle).
1.6.3 Calcium Imaging in Basket Cell Dendrites

Also, there have been several studies that, collectively, have shown that dendritic calcium signal recordings are predictive of dendritic spike amplitudes in hippocampal CA1 basket cells as well (reviewed in Hu et al, 2014). Specifically, basket cell dendritic action potential amplitudes follow a sharp continuous decline and are usually less than half their original amplitude by about 75 µm (Hu et al, 2010). These results are explained by basket cells having very little dendritic sodium channels with almost no sodium channels at distances greater than 100 µm as well as high potassium to sodium channel ratios. Functionally, this seems to have the effect of accelerating the decay time course of excitatory postsynaptic potentials, which shortens the time period of temporal summation and promotes action potential initiation with high speed and temporal precision (Hu et al, 2010). Similarly, dendritic calcium signals in CA1 basket cells decrease continuously and are no longer observable by about 50 µm along the dendrites (figure 1, right; Camiré and Topolnik, 2014). In summary, these three cases support that calcium signal decay can be highly predictive of backpropagating action potential amplitude decay.

Figure 1. Interpretation of dendritic calcium signal recordings. Pyramidal Cells: In the top plot we see backpropagating spike amplitude decay along the dendrites of the first spike in 10-20Hz spike trains, elicited at the soma. Past 300 µm spike amplitude decreases in weak propagating
neurons, but does not decrease in strong propagating neurons. In the bottom plot we see somatic action-potential-induced intracellular calcium signal changes along the dendrites, normalized to the maximum fluorescence change. Specifically, weak propagating neurons (filled data points and dashed lines) show strong decreases in calcium signals whereas strong propagating neurons (empty data points and solid lines) do not show strong decreases in calcium signals. (both pyramidal cell figures taken from Golding et al, 2001). OLM Cells: In the top plot we see the ratio of dendritic and somatic action potential amplitudes at different points along the dendrites, with very little decreases in amplitude with distance from soma (empty circles, triangles and squares; taken from Martina et al, 2000). In the presence of TTX (filled circles) we see a strong decline in action potential amplitude with distance from soma. In the bottom plot we see action potential evoked calcium transients in the dendrites of putative OLM cells, again with only mild decreases in calcium signal amplitude with distance from soma (taken from Topolnik et al, 2009, supplemental material). Basket Cells: In the top plot we see a strong decrease in the first (red) and last (blue) action potential amplitudes with distance from soma during 1s spike trains (taken from Hu et al, 2010). In the bottom plot, we see a similar trend in the amplitude of backpropagating calcium signal transients evoked by trains of 10 somatic action potentials (mean = red; dotted line = threshold of signal detection; taken from Camirè and Topolnik, 2014)

1.7 Single Neuron Modeling

Although there are many types of modeling techniques that are available for modeling single cells, in this section we will focus on the techniques available for modeling morphologically detailed multi-compartment models. These techniques involve the use of cable theory and conductance based modeling for generating biophysically realistic, experimentally constrained predictions of single cell active properties.

1.7.1 Cable Theory and Multi-Compartment Modeling

Because of the electrically conducting nature of neural processes, they can naturally be described as core conductors using cable theory (reviewed in Rall, 1977; Holmes, 2010). Cable theory was first applied to the first transatlantic telegraph cable and has proven to be very applicable to studying the complex processes found in neurons, be it either to study nonlinear passive properties or active membrane properties (e.g. VGCs). Passive modeling using cable theory relies on parameters such as specific membrane capacitance ($C_m$), specific membrane resistance
(R_m), specific membrane conductance (G_m) and axial resistance (R_a) to model passive cellular properties such as input resistance (R_N), membrane time constant (τ_m) and resting membrane potential (V_r). Mathematically this can be conceptualized by applying Ohm’s law and Kirchoff’s law. For example, in the case of a circuit for a one-dimensional neuron cable we have the following partial differential equation:

\[ \tau \frac{\partial V}{\partial t} = \lambda^2 \frac{\partial^2 V}{\partial x^2} - V \]

\[ \tau = R_m G_m \]

\[ \lambda = \frac{R_m d}{\sqrt{4R_a}} \]

This essentially describes how the change in intracellular current is equal to the current flowing across the membrane in a one-dimensional cable (Holmes, 2010). In this case, \( \tau \) is the membrane time constant, \( \lambda \) is the membrane space constant, \( d \) is the diameter of the cylinder, and \( V \) is the voltage, which is dependent on both time \( (t) \) and distance along the length of the core conductor \( (x) \).

In order to use passive cable theory as a basis for multi-compartment modeling, the partial differential equation describing the cable is discretized (Rall, 1964; De Schutter and Van Geit, 2010; Carnevale and Hines, 2006). In other words, these discretized versions of the partial differential equations represent the flow of current across the morphology of a neuron in the form of compartments. For example, for a one-dimensional cable we use the following equation:

\[ c_{m_j} \frac{dV_j(t)}{dt} = \sum_k \frac{V_k(t) - V_j(t)}{r_{a_{jk}}} - I_{ionic,j} \]

\[ s_j = \pi d_j l_j \]

\[ c_{m_j} = C_m s_j \]

\[ r_{a_{jk}} = \frac{4R_a l_j}{\pi d_j^2} \]
The first term on the right side of the first equation describes the flow of axial current between compartments $j$ and $k$. The second term, $I_{ionic,j}$, describes any active membrane currents in compartment $j$, such as from VGCs. Regarding additional variables, $s_j$ is the surface area of the membrane of compartment $j$, $d_j$ is the diameter of compartment $j$, $l_j$ is the length of compartment $j$, $c_m$ is membrane capacitance of compartment $j$ and $r_{ajk}$ is the cytoplasmic resistance.

1.7.2 Hodgkin-Huxley Formulation for Modeling Channel Kinetics

In this section we will elaborate on the $I_{ionic}$ term, specifically in regards to the current generated from VGCs embedded in the cellular membrane of neurons. Most commonly, VGCs are modeled using conductance-based modeling approaches, which traditionally follow Hodgkin-Huxley formulation. This is a simple but effective approach that relies on measurements of voltage-sensitive channel gating kinetics (e.g. activation, inactivation and deactivation) to represent ion channels as conductances. Alternatively, one can also use a more accurate Markovian formulation, which is based on state diagrams that are analogous to the conformational changes of channels. Since Markovian formulation is typically only used when particular channel-specific parameters have been obtained (i.e. channel measurements that are not yet available for IS3 cells), we will focus on Hodgkin-Huxley formulation (Destexhe and Huguenard, 2010).

The original Hodgkin-Huxley model is based on experimental data from the giant squid axon (Hodgkin and Huxley, 1952). In this experiment, voltage clamp was used to characterize the kinetics of two voltage-dependent currents (i.e. $I_{Na}$ and $I_K$). The voltage dependence of these channels was captured in a mathematical model, which was developed to demonstrate that the voltage-dependent kinetics of the two channels were sufficient to explain how action potentials were being generated. The mathematical model is summarized here:

$$I_{Ionic} = g_L(V - E_L) + \tilde{g}_{Na} m^3 h (V - E_{Na}) + \tilde{g}_K n^4 (V - E_K)$$

Where the first term is the leak current term, the second term is the sodium current term and the third term is the potassium current term. Furthermore, $g_L$ is the membrane conductance for leak currents, $\tilde{g}_{Na}$ and $\tilde{g}_K$ represent the maximal conductance values generated from sodium and potassium currents and $E_L$, $E_{Na}$ and $E_K$ represent the reversal potentials for leak currents, sodium
currents and potassium currents. Essentially, both sodium and potassium currents are controlled by the voltage-dependent gating kinetic variables, $m^3$, $h$ and $n^4$:

\[
\begin{align*}
\frac{dm}{dt} &= \alpha_m (1 - m) - \beta_m m \\
\frac{dh}{dt} &= \alpha_h (1 - h) - \beta_h h \\
\frac{dn}{dt} &= \alpha_n (1 - n) - \beta_n n
\end{align*}
\]

\[\begin{align*}
\alpha_m &= \frac{-0.1(V - V_r - 25)}{\exp\left[-\frac{V - V_r - 25}{4}\right] - 4} \\
\alpha_h &= 0.07 \exp\left[-\frac{V - V_r}{20}\right] \\
\alpha_n &= \frac{-0.01(V - V_r + 10)}{\exp\left[-\frac{V - V_r + 10}{10}\right] - 1} \\
\beta_m &= 4 \exp\left[-\frac{V - V_r}{18}\right] \\
\beta_h &= \frac{1}{1 + \exp\left[-\frac{V - V_r - 30}{10}\right]} \\
\beta_n &= 0.125 \exp\left[-\frac{V - V_r}{80}\right]
\end{align*}\]

Where the forward ($\alpha$) and backward ($\beta$) rate constants (i.e. the transitions back and forth between two states) of $m$ and $n$ promote channel activation during depolarization, whereas the rate constants of $h$ promotes closing of the gate during depolarization (i.e. inactivation). In other words, it was found that sodium channel kinetics could be explained using three activation gates ($m^3$) and one inactivation gate ($h$), whereas potassium channels kinetics could be explained using four activation gates ($n^4$) and no inactivation gates. Note that $V_r$ is the resting membrane potential and that the rate constants were estimated by fitting voltage-dependent functions to the experimental data (Hodgkin and Huxley, 1952; Destexhe and Huguenard, 2010).

Furthermore, this formalism can be rewritten in a form that is more convenient for fitting to experimental steady-state activation/inactivation ($m/\alpha$) and activation/inactivation time constant ($\tau_m/\tau_h$) curves (Destexhe and Huguenard, 2010). For example, in the case of $m$:
Using this formalism, the process of fitting experimental channel kinetic data to mathematical Hodgkin-Huxley equations can be simplified (Destexhe and Huguenard, 2010). In turn, this has allowed the development of many biophysically based channel models of varying ionic types and gating kinetics that are often specific to particular cell types.

1.7.3 Previous Morphologically Detailed Hippocampal Interneuron Models

Although multi-compartment modeling has been used to model a variety of different cell types across the brain, we will focus here on several examples of morphologically detailed hippocampal interneuron models where specific biological constraints were applied to the models. There are numerous examples of hippocampal interneuron models (Skinner and Ferguson, 2015). However, we will mainly discuss OLM and basket cell multi-compartment models, as these hippocampal interneuron types have models that were derived from morphological reconstructions. Particularly, we will describe different strategies in developing multi-compartment models as well as different ways in which the models were used and tested to generate experimentally constrained predictions. In doing this, we will also illustrate different contexts in which it was appropriate to have reduced amounts of morphological detail versus contexts in which it was appropriate to have full morphological models (e.g. based on the number of compartments, inclusion of dendritic branches, inclusion of axon).

1.7.3.1 OLM Cell Morphologically Detailed Models

The first morphologically detailed multi-compartment model of a hippocampal interneuron was the OLM cell. It was first developed using an OLM cell morphological reconstruction from CA1 in order to explore the active properties of OLM cell dendrites (Saraga et al, 2003). In this model, the passive parameters were hand-tuned within biologically realistic ranges so as to
match the model with experimental OLM measurements of input resistance and membrane time constant. Preliminary somatic and dendritic VGC types and densities were then chosen so as to replicate spiking patterns that are seen experimentally in OLM cells. The resulting OLM cell model was then used to make predictions regarding action potential initiation and propagation throughout the dendrites. Since this work focused on the dendritic properties of OLM cells and little was known about OLM axon properties, it was appropriate to use a reduced morphology (i.e. containing only soma and dendrites to reduce computational strain) where the axon was either removed or a simple representation of an axon/axon initial segment was included.

In an additional study, a more developed biologically constrained OLM cell multi-compartment model (i.e. with new reconstructions and additional channel currents) was used to predict that M-current (composed of Kv7 subunits) likely followed a somatodendritic distribution in OLM cells (Lawrence et al, 2006). Importantly, this model was also further constrained by specific types of calcium and potassium channels (i.e. as opposed to the preliminary set of channels used in Saraga et al, 2003). Similar to Saraga et al, 2003, this model was developed using a reduced axonal morphology.

More recently, databases of morphologically detailed multi-compartment models of OLM cells (i.e. with varying channel conductance balances) were generated through the use of ensemble modeling techniques in order to predict the important channel balances and distributions that control OLM cell activity (Sekulić et al, 2014). In this case, the channel types used in these models were similar to the channel types found in Lawrence et al, 2006, and therefore possessed similar biological constraints. In particular, this study looked at the importance of dendritic h-current in generating appropriate electrophysiological activities that are seen in experimentally recorded OLM cells. Moreover, this approach revealed two channel co-regulations that are dependent on the presence of dendritic h-current, which suggests that dendritic h-current plays an important role in generating OLM cell electrophysiological activity. Similar to previous OLM cell models, this model was developed using a reduced axonal morphology.

A follow-up study using highly ranked models from Sekulić et al, 2014, further revealed that non-uniform (i.e. both linear and sigmoidal) decreasing dendritic densities of h-current with distance from soma as well as adjusted h-current activation kinetics could more optimally replicate experimental hyperpolarization recordings from OLM cells (Sekulić et al, 2015). More
specifically, optimization procedures were used to optimize both the h-current activation parameters as well as the parameters of several potential distributions (i.e. uniform, linear and sigmoidal) of h-current along OLM dendrites. The procedure used was a minimization of the sum of squared errors between the model and experimental voltage traces. Although optimized distribution parameters indicated a preference for decreasing h-current densities with distance from soma, this result was morphology dependent, where the smaller OLM cell multi-compartment model (i.e. by surface area and dendritic compartment length) showed more of a preference for uniform h-current distributions. Furthermore, dendritic distributions of h-current also appeared to increase the backpropagation speeds of action potentials along OLM model dendrites. These findings strongly motivated further experimental studies. Morphology and recordings from the same cell with and without h-currents blocked have now been obtained for several OLM cells to allow examination of these findings.

1.7.3.2 Basket Cell Morphologically Detailed Models

In addition to hippocampal OLM cell models, there are several examples of morphologically detailed hippocampal basket cell models from both dentate gyrus and CA1. Here we will discuss these examples as well as their varying degrees of morphological detail, depending on the biological context being investigated.

In one study (Santhakumar et al., 2005) a 527 cell network model of dentate gyrus was constructed using biophysically realistic, morphologically detailed cell models of mossy cells, granule cells, basket cells and hilar cells. Specifically, the purpose of this network model was to investigate the effects of mossy cell loss and mossy fiber sprouting on dentate gyrus hyperexcitability. Respectively, the model demonstrated that mossy fiber sprouting resulted in the spread of seizure like activity and loss of mossy cells resulted in reduced responses from granule cells. In this case, the morphology of the basket cell model was represented by a soma and four dendrites, each possessing four compartments (i.e. a total of 17 compartments). Because of the network context and the presence of conductance-based VGCs in these models, it was appropriate to use reduced morphological schematics. This effectively reduced the computational strain, while still being able to investigate the morphological effects of mossy cell sprouting on dentate gyrus hyperexcitability.
In another study (Saraga et al., 2006), a more detailed morphological multi-compartment model of a two-cell basket cell network was developed (i.e. each cell had one somatic and 371 dendritic compartments). The purpose of this model was to investigate the effect of dendritic gap junctions on network output when varying levels of active dendritic properties are present. In this case, both gap junction densities on distal dendrites and dendritic active properties had modulatory effects on network dynamics. Although the morphology was more detailed than previous attempts, this model was based on morphological observations of CA1 basket cells taken from the web (i.e. the model was not developed using a full morphological reconstruction). Given the context of this work, however, this was a reasonable approach since the goal was to investigate network outputs from simplified electrical dendritic connection schemes (i.e. an approximate basket cell morphology was sufficient for this).

Another group (Nörenberg et al., 2010) developed a full morphology multi-compartment model (i.e. complete with soma, dendrites and axon) to investigate the passive properties of dentate gyrus basket cells. Specifically it was found that the unique non-uniform passive parameter distributions (specific membrane capacitance, specific membrane resistance and axial resistivity) of basket cells enable the acceleration of synaptic potential time courses at the soma in response to fast inputs and increase the efficacy of slow distal inputs. In a subsequent study (Hu et al., 2010) this full morphology multi-compartment model was used to investigate the high potassium to sodium channel ratio found in the dendrites of dentate gyrus basket cells. As mentioned previously (i.e. section 1.6.3), this study found that dendritic potassium densities increased the speed and temporal precision of excitatory activation in basket cells. In another subsequent study (Elgueta et al., 2015), mechanisms of persistent firing (i.e. long-lasting spike trains that persist following the removal of a stimulus) in dentate gyrus basket cells were investigated using the same full morphology multi-compartment model. In this case, it was shown that hyperpolarization-activated current could contribute to the generation of persistent firing by shifting its activation curve towards more depolarized regimes. In all three of these cases, the use of a full morphology multi-compartment model was essential in generating specific biophysically realistic predictions. Essentially, these studies made predictions that purely focused on the intrinsic properties of basket cells and the use of full morphology multi-compartment models allowed a higher degree of exploration and precision.
1.8 Thesis

1.8.1 Rationale

Morphological and synaptic aspects of IS3 cells are being examined, but what type, how much and where VGCs are present has not been determined. As other hippocampal interneuron types are known to have high densities of VGCs on their dendrites (Hu et al., 2010; Martina et al., 2000), it seems likely that this could be the case for IS3 cells also. As mentioned, it has been shown that there is only a small decline in action potential evoked calcium signals in the dendrites of OLM cells (Topolnik et al., 2009). Similar preliminary results have been shown in IS3 cells, which suggest that IS3 cells might possess balances of VGCs on their dendrites that promote action potential backpropagation, as it is known that this is the case for OLM cells. Determining and understanding particular characteristics of specific cell types is a highly challenging endeavour using purely experimental means. Thus, we have begun development of IS3 computational cell models to help address this challenge.

1.8.2 Hypothesis

Because of IS3 to OLM weak synaptic transmission properties, we assume that IS3 cells need to reliably elicit a spike when activated (i.e. only require a small threshold to fire) so that it is more likely that they can fire synchronously and exert control over OLM cells when activated. We hypothesize that dendritically located VGCs are important for IS3 cells to fire reliably.

Since channel types, densities and distributions in IS3 cells are currently unknown, we need to estimate these biophysical properties. We aim to do this by developing multi-compartment models that mimic voltage dynamic profiles expressed by IS3 cells. These developed models in turn can provide predictions regarding the biophysical properties of IS3 cells. Then, to investigate our hypothesis we analyze spike reliability in the most appropriate IS3 models.

Essentially, the models generated here will provide guidance for experimentalists in narrowing down which biological details of IS3 cells are both plausible and important. It is generally expected that certain characteristics (e.g. VGCs on the dendrites of IS3 cells) will be necessary for the IS3 cell to fire reliably enough (i.e. by minimizing the synaptic weight and synaptic distance from soma necessary to elicit somatic spikes) to exert its influence on OLM cells.
1.8.3 Goals

1) **Develop** an approach to strategically estimate appropriate channel types, channel conductance ranges, channel combinations and channel distributions in morphologically reconstructed multi-compartment models of IS3 cells.

2) **Use** the developed approach to generate models that match IS3 characteristics

3) **Test** the firing reliability of models that generated appropriate outputs
2 Methodology

2.1 Experimental Data

Note that all experiments are performed by Olivier Camiré in Dr. Lisa Topolnik’s laboratory at Université Laval in Québec City.

2.1.1 Slice Preparation

Transverse hippocampal slices (300 µm) are prepared from VIP/enhanced green fluorescent protein (VIP-eGFP) mice of either sex (postnatal day 14-23) as described previously (Tyan et al, 2014) in accordance with the animal welfare guidelines of Université Laval.

2.1.2 Electrophysiological Recordings

VIP-eGFP cell are identified and patch clamp recordings are performed as described previously (Tyan et al, 2014). Slices are perfused with standard artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2 MgSO4, 2 CaCl2, and 10 glucose saturated with 95% O2 and 5% CO2, pH 7.4, at 30 –33°C. For current clamp recordings, the intracellular solution contains (in mM): 130 KMeSO4, 2 MgCl2, 10 diNa-phosphocreatine, 10 HEPES, 2 ATP-Tris, 0.2 GTP-Tris and 0.3% biocytin (Sigma), pH 7.2– 7.3, 280–290 mOsm/L. To reduce synaptic activity during recordings, gabazine (1 µM) as well as glutamate receptor antagonists L-AP5 (100 uM) and NBQX (10 uM) are also applied.

2.1.3 Two-Photon Calcium Imaging

Dendritic calcium signal recordings are performed as described previously (Camiré and Topolnik, 2014). Using the patch electrode, cells are filled with Oregon Green® 488 BAPTA-1 (OGB-1) calcium indicators for 20-30 min before imaging. Dendrites are identified using red fluorescence from Alexa Fluor 594. Calcium signals are measured by collecting green and red fluorescence during 500 Hz line scans across dendritic segments of 2-15 µm. Fluorescence changes are quantified as changes in green fluorescence from the baseline ΔF/F = (F - F0)/F0.
2.1.4 Morphological Reconstructions and Neurochemical Analysis

Morphological reconstructions and neurochemical analysis of IS3 cells are performed as described previously (Tyan et al., 2014; Camiré and Topolnik, 2014). Recorded neurons are filled with biocytin (Sigma) during whole-cell recordings. These are treated to reveal the biocytin so that z-stacks can be acquired (in 1 µm steps) and antibody labeling can be performed. Morphological reconstructions are traced from the z-stacks using the Neurolucida 8.26.2 software (MBF Bioscience) and the expression of specific markers is revealed through confocal images of the antibody labeled sections.

2.2 Computational Modeling

All simulations are performed and analyzed using a 2010 Macbook Pro with the OSX Yosemite platform, an Intel® Core™ i7-3770 CPU @ 3.40GHz × 8 with the Ubuntu 14.04 LTS platform or the Neuroscience Gateway (NSG) for high performance computing (Sivagnanam et al., 2013).

2.2.1 Simulation and Analysis Software

The model is developed and simulated in version 7.3 of the NEURON software environment (Carnevale and Hines, 2006) on all of the platforms listed previously. All simulations as well as experimental recordings are analyzed using a MATLAB toolbox called PANDORA (Günay et al., 2009) as well as customized MATLAB code.

2.2.2 Morphology/Compartmentalization

Topological information provided by the morphological reconstruction of an IS3 cell is imported directly into NEURON using NEURON’s CellBuilder graphical user interface. The morphological structure is then subdivided into multiple compartments until the point where the compartments are small enough to be considered isopotential.

2.2.3 Passive Properties

The model’s passive parameters are adjusted, such that resting membrane potential, input resistance and membrane time constant are matched with their appropriate experimentally observed values. In this case, the model’s passive properties are matched with its respective cell’s raw data and not the experimental averages. Also, parameters such as specific membrane capacitance and axial resistance are restricted to appropriate experimentally estimated ranges.
For example, the average measurement of specific membrane capacitance in hippocampal interneurons is 0.9 ± 0.3 uF/cm² (Chitwood et al., 1999). As well, the average measurement of specific membrane capacitance in cortical pyramidal neurons, spinal cord neurons and hippocampal neurons is also 0.9 uF/cm² (Gentet et al., 2000), suggesting that the range for this parameter is fairly consistent across many neuron types. Similarly, experimental ranges of axial resistance also exist but are generally more flexible. For example, some sources state that values for axial resistance ranges in individual neurons range between 100-300Ω•cm (Dayan and Abbott, 2005), whereas other sources state a more flexible range between 50-400Ω•cm (Holmes, 2010).

2.2.4 Active Properties

2.2.4.1 Develop: Parameter Search Investigation By Automation and Retrofit Hand-Tuning (PSIBAuRg)

As would be expected, it is in this section where the bulk of the work lies, since it is here where predictions are made regarding what channel types are likely to be found in IS3 cells. To do this, I have developed an approach called PSIBAuRg: Parameter Search Investigation By Automation and Retrofit Hand-Tuning. In this sense, it combines hand tuning with automation in order to allow both automated simulations of multiple electrophysiological features simultaneously and manual modeling based on knowledge of the kinetic functions of channel types. The steps of this approach are outlined here:

**Automate** the generation of a database of models and test them at multiple current injection protocols (i.e. CIPs: -100pA, 20pA, 50pA and 500pA). Note that before the first round of automated simulations, appropriate channel conductance parameter spaces are estimated through hand tuning in order to establish a starting point. As mentioned, the models are generated in the NEURON software environment (Carnevale and Hines, 2006) and each model in the database possesses a unique combination of channel conductance values. The CIPs chosen here are intended to represent the key signature features seen in experimental IS3 recordings (see table 1 and figure 2). The voltage traces generated from each model are then imported into MATLAB using PANDORA (Günay et al., 2009) and organized into databases.

**Eliminate** the models that do not capture IS3 features at the given CIPs. In general, this step is referred to as the “pre-processing” step (see table 1) because (in MATLAB) it automatically edits
out any clearly inappropriate models (e.g. if a model spikes at 20pA, remove that model) before further evaluations of the database can be performed.

**Measure** the electrophysiological characteristics of the remaining models. Specifically, each CIP possesses its own set of characteristic measurements (e.g. sag in membrane potential for the -100pA CIP or spike rate in the 50pA CIP). See table 1 for the full list of measurements. Although most of these measurements are included in the PANDORA toolbox, two customized measurements were added to the toolbox functions (i.e. membrane potential difference and membrane potential difference in the last 700ms of the CIP).

**Compare** the model measurements against those seen in the experimental IS3 traces. For this step, experimental traces (i.e. figure 2) are also imported into PANDORA where they are analyzed using the same measurements as those used to analyze the models. The Euclidean distance between each model and the canonical experimental trace (as seen in figure 2) is then computed:

\[ d_{x,y} = \sum_{i=1}^{N} \frac{|x_i - y_i|}{N} \]

In this case, \( x_i \) and \( y_i \) represent the \( i \)th measurement out of \( N \) total measurements for the simulation and experimental traces, respectively. In this sense, \( d_{x,y} \) generally serves as a quality metric for each of the models.

**Rank** the models to find the best models in the remaining database, in order of smallest Euclidean distance to largest (i.e. best to worst).

**Adjust** the model parameter space, distributions or channel types manually to improve the number of appropriate models and individual model measurements. This step generally involves hand tuning the model in several ways. For one, clutter based dimensional reordering (CBDR) plots (figure 4 and 8, right; Taylor *et al*, 2006) are used to visualize the parameter space so that we can then adjust it so as to maximize the number of appropriate models in the database. Specifically, these plots visualize the structure of the conductance parameter space by reordering the channel conductance parameters along an N-dimensional tensor in order to sort higher order
channels from lower order channels (i.e. channels that have more and less of an impact on Euclidean distance, respectively). Therefore, in order to minimize space in the plots where models had been rejected (i.e. the black pixels in figures 4 and 8) we adjust the next database to focus only on conductance parameter spaces that had generated good models (i.e. by adjusting the conductance resolution and range for each channel). Secondly, different cases are analyzed, where each case possesses a different distribution of channels along the IS3 morphology (table 2). Finally, decisions are made regarding whether certain channels should be added, altered or removed in order to improve the model. These decisions are made in a variety of ways. For example, if a certain channel is likely to worsen a particular measurement in the model, then that channel is removed. Likewise, if hand tuning showed that a channel is likely to contribute well to the model’s measurements, then that channel is included in the model. As well, the Allen Mouse Brain Atlas (Lein et al, 2007) is also used to analyze whether particular channel subunits are present in the CA1 SR layer (i.e. where IS3 cell soma and dendrites are mainly located). This usually indicates whether or not a particular channel is likely present in IS3 cells and also carries implications regarding specific channel kinetics depending on the channel subunit compositions found in the SR layer.
Table 1. Summary of steps used to identify appropriate IS3 models using the PSIBAuRg approach.

<table>
<thead>
<tr>
<th>CIP Amplitude</th>
<th>Signature Feature</th>
<th>Characteristic measurements during CIP*</th>
<th>Pre-processing (Eliminate Model…)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-100 pA</td>
<td>Lack of “sag”</td>
<td>-Sag in membrane potential</td>
<td>-None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Sag decay time constant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Time at which minimum in membrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>potential occurs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Minimum membrane potential</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average membrane potential difference** (pulse-initial)</td>
<td></td>
</tr>
<tr>
<td>20 pA</td>
<td>No Spiking</td>
<td>-Average membrane potential difference** (pulse-initial)</td>
<td>-If spikes</td>
</tr>
<tr>
<td>50 pA</td>
<td>Normal Spiking</td>
<td>-Average spike threshold</td>
<td>-If less than 3 spikes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average spike amplitude</td>
<td>-If membrane potential fails to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average interspike interval</td>
<td>repolarize</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average spike half-width duration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Latency of first spike</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average spike afterhyperpolarization</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Number of spikes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Spike rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Spike frequency accommodation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average membrane potential difference** (pulse-initial)</td>
<td></td>
</tr>
<tr>
<td>500 pA</td>
<td>Depolarization Block</td>
<td>-Spike rate in first 100ms of current injection</td>
<td>-If spikes in last 700 ms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Average membrane potential difference** (pulse-initial) in the last 700ms</td>
<td>-If membrane potential fails to repolarize</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-If spikes in recovery period</td>
</tr>
</tbody>
</table>

*CIP = Current Injection Protocol

**Average membrane potential difference = the difference between the average membrane potential before the CIP versus during the CIP
Table 2. Summary of channel type combinations and spatial distribution profiles across the morphology of the model. Note that in all cases, each channel has a uniform distribution, whether it be restricted to the soma or distributed across the soma and dendrites.

<table>
<thead>
<tr>
<th>Case #</th>
<th>Soma Channel Types</th>
<th>Dendrite Channel Types</th>
<th>Axon Channel Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>Persistent Sodium</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Transient Sodium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Type Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 2</td>
<td>Persistent Sodium</td>
<td>Transient Sodium</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Transient Sodium</td>
<td>A-Type Potassium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Type Potassium</td>
<td>Fast Delayed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast Delayed Rectifier Potassium</td>
<td>Rectifier Potassium, Slow Delayed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>Persistent Sodium</td>
<td>Transient Sodium</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Transient Sodium</td>
<td>Fast Delayed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Type Potassium</td>
<td>Rectifier Potassium, Slow Delayed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slow Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 7</td>
<td>Persistent Sodium</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Transient Sodium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Type Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Faster Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 8</td>
<td>Persistent Sodium</td>
<td>Transient Sodium</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Transient Sodium</td>
<td>A-Type Potassium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Type Potassium</td>
<td>Faster Delayed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Faster Delayed Rectifier Potassium</td>
<td>Rectifier Potassium</td>
<td></td>
</tr>
<tr>
<td>Case 9</td>
<td>Persistent Sodium</td>
<td>Transient Sodium</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Transient Sodium</td>
<td>Faster Delayed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Type Potassium</td>
<td>Rectifier Potassium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Faster Delayed Rectifier Potassium</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2.4.2 Use: Exploring Non-Uniform Channel Distributions

Using the PSIBAuRg approach, appropriate channel types, distributions and balances are identified. However this approach only utilizes the experimental voltage recordings of IS3 cell somata and does not consider the experimental results obtained from dendritic calcium imaging recordings. In fact, dendritic calcium signal recordings provide important insight towards the potential presence of channels across the dendrites of IS3 cells. For this reason, dendritic action potential propagation is analyzed in the most appropriate models in order to compare with the calcium signal propagation observed in IS3 cell recordings. Specifically the amplitudes of the propagated spikes along the dendrites of the model are compared with the experimental calcium signal amplitudes. Because calcium signal deterioration is not necessarily indicative of action potential decay, this form of analysis mainly predicts that dendritic VGCs in at least the first 50 µm of dendrites are necessary for action potential propagation (i.e. since there is no calcium signal deterioration in these first 50 µm of IS3 dendrites).

2.2.4.3 Test: Model Usages

2.2.4.3.1 Irregular Firing Analysis in Top Models

Since there is evidence that irregular firing when held close to threshold is a prominent feature in experimental recordings of IS3 cells, it is also important to show that the best models generated here are capable of showing irregular firing patterns. Since we know from previous work (i.e. Stiefel et al, 2013) that irregular firing is largely reliant on fast time constants in delayed rectifier potassium channels (i.e. for interneurons at least) and noise magnitude, we include fast delayed rectifier potassium channels in our model and focus on the effect of noise magnitude on irregular firing. Like in Stiefel et al (2013) we quantify irregular firing using the coefficient of variation of the interspike interval (ISI CV):

\[ ISI \text{ CV} = \frac{ISI \text{ SD}}{ISI \text{ mean}} \]

Also similar to Stiefel et al, 2013, we consider spike patterns irregular when the ISI CV is above 1 and regular when the ISI CV is below 0.5. Specifically, we look at current injection ranges between already established non-spiking regimes (0.02nA) and regular spiking regimes (0.05nA) using intervals of 0.0005nA. For each current injection, we test the model with somatic injections of gaussian noise standard deviations ranging from 0.01nA to 0.1nA. Note that during
the PSIBAuRg approach the models are always generated using minimal somatic gaussian noise (0.01nA). This therefore allows us to look at the effect of gaussian noise magnitude on the ISI CV of each model as each model transitions from non-spiking to regular spiking (i.e. the range where spiking is supposedly expected to be irregular). Note that these tests are performed on the top models that were generated using the PSIBAuRg approach and they are performed using the NSG.

2.2.4.3.2 Firing Reliability Analysis in Top Models

Here we describe spike reliability as the relationship between synapse distance from soma and the resultant synaptic weight necessary to elicit a somatic spike. Once several cases of appropriate models are generated, top models are then analyzed in regards to spike reliability. This test is done using NEURON’s NetCon class to define an AMPA synaptic connection that delivers a single presynaptic spike to a target point along the model’s dendritic tree. In this case, the cells synapse is defined using the Exp2Syn point process. This point process describes the synapse as a two state kinetic scheme to produce synaptic current according to the following:

\[
G = \text{weight} \times \text{factor} \times \left( \exp \left( -\frac{t}{\tau_2} \right) - \exp \left( -\frac{t}{\tau_1} \right) \right)
\]

\[
i = G(v - e)
\]

Where \(i\) is the synaptic current (nA), \(G\) is the synaptic conductance (uS), \(v\) is the membrane potential (mV), \(e\) is the reversal potential (set to 0 mV), \(\text{weight}\) is synaptic weight (uS) as specified by the NetCon object, \(\text{factor}\) is a NEURON process used to normalize the peak synaptic conductance to 1, \(\tau_1\) is the rise time (set to 0.2 ms) and \(\tau_2\) is the decay time (set to 2 ms). Although rise time and decay time are fairly variable across different cell type pairs (e.g. these values are quite different between granule cell to pyramidal cell connections and granule cell to inhibitory cell connections), the values chosen here are chosen to reflect approximately appropriate values based on measurements of these values in other interneurons in hippocampus (Tóth, 2010). This synaptic model is then incrementally moved to different locations along the dendrite. At each location, the minimum \(\text{weight}\) value necessary to evoke an action potential at the soma is numerically determined by increasing the weight in increments until an APCount point process located at the soma reported that the membrane potential had surpassed -20 mV in response to a single presynaptic spike. After a somatic spike is
recorded, the synapse is then moved to a further dendritic location and the process is repeated until the entire dendritic arbor is analyzed.

Importantly, reliable firing in this case is characterized by how large the threshold weight is in proportion to how far the synapse is from the soma. For example, if a synapse that is far away from the soma only requires a small weight conductance to evoke an action potential at the soma, then that model is considered to fire more reliably than a model that requires a large weight conductance at the same location.

2.2.4.3.3 Spike Time Precision in Top Models

Finally, irregular firing near threshold suggests that minimally activated IS3 cells may possess more variable spike times when receiving weak excitatory inputs. This, in turn, may reduce the ability of IS3 cells to elicit closely timed action potentials while in this regime. To test whether irregularly firing regimes have a large effect on the precision of spike time, we look at the effect of intrinsic noise magnitude on spike time precision when synaptic weight ranges are near threshold. Similar to the spike reliability tests, a single presynaptic event activating an AMPA receptor is modeled on the somas of the top models (i.e. using NEURON’s NetCon class and the Exp2Syn point process). For each model, the synaptic weight and the intrinsic noise magnitude are augmented incrementally and for each combination, the somatic spike time resulting from the single presynaptic spike is recorded using NEURON’s APCount point process. This 20ms simulation is repeated 10 times for each weight and noise combination so that the standard deviation of spike times from each top model can be analyzed.
3 Results

3.1 Experimental Data

3.1.1 IS3 Electrophysiological Regimes during Current Injections

In order to develop a strategic approach for estimating IS3 channel types, densities and distributions, we first examine electrophysiological data of IS3 cells obtained by Dr. Lisa Topolnik’s lab group at Université Laval. From the current clamp voltage traces, we are aware of several IS3 electrophysiological features (figure 2). For one, during hyperpolarization, IS3 cells exhibit little membrane potential sag (figure 2, 1st current injection). As well, we also know that during depolarizing currents, IS3 cells generally do not start firing until around the 20-30pA mark (figure 2, 2nd current injection). In terms of firing dynamics, IS3 cells tend to demonstrate irregular firing patterns when held close to threshold, with occasional demonstrations of regular adaptive firing (i.e. usually when held at around 30pA). At higher current injections (i.e. from about 30-50pA), firing is, in general, more regular (i.e. smaller ISI CVs). Furthermore, at higher current injections, progressive spike amplitude adaptation is observed (i.e. decreasing spike amplitudes during current injections), with higher current injections having larger decreases in amplitudes during the current injection (figure 2, 3rd current injection). Finally, by around 250 pA, the cell goes into depolarization block where you generally see only one spike at the beginning of the current injection followed by a flattening of the trace (figure 2, 4th current injection).
Figure 2. Experimental IS3 traces during current clamp showing IS3 electrophysiology at -100pA, 20pA, 50pA and 500pA (from left to right, respectively). Recordings are done in the presence of synaptic blockers (i.e. NBQX, AP5 and Gabazine).

3.1.2 IS3 Calcium Signal along Dendrites

In order to get a sense of the dendritic distributions of voltage-gated channels, we examine dendritic two-photon calcium imaging data of IS3 cells provided by Dr. Lisa Topolnik’s lab group at Université Laval. In these experiments, three consecutive high amplitude pulse currents (800 pA, 2 ms durations) are injected into the soma and resulting calcium transient amplitudes are recorded at different points along the dendrites. Preliminary IS3 dendritic two-photon calcium imaging reveals that calcium signals along the dendrites are non-degrading up until roughly 50 µm from the soma. From then on, they can still be seen reliably up until roughly 150 µm from the soma. Although considerable variability is observed between recordings from individual IS3 cells, all the IS3 cells follow the same trend with distance from soma and there is a statistically significant decline in calcium signal between 50 µm and 150 µm (i.e. using a signed-rank test; unpublished observations, Topolnik Lab). Although these experimental calcium signal recordings are indicative of calcium activity and not necessarily action potential
propagation and amplitude decay, we assume, based on previous work (see section 1.6), that dendritic calcium signal amplitudes are predictive of dendritic action potential amplitudes. In other words, since we see calcium signals at large distances from the soma in IS3 cells, it is assumed that VGCs must be present to propagate the action potential and activate calcium channels.

3.2 Computational Modeling

3.2.1 Morphology/Compartmentalization

The topological model is shown in figure 3 (bottom). Because inclusion of the full axon in the model requires more specifics regarding biophysical axonal properties (i.e. channels types and distributions) and more computational strain, we consider two morphological versions of the model: one where the axon is cut down to a small remaining section (M2) and one with the axon intact (M1). In the M2 version, the number of compartments is reduced from 653 to 221 and the surface area is reduced from 20,871 µm² to 7,297 µm² (approximately 35% of the original surface area). While both morphologies are considered, we focus more so on the M2 version.
3.2.2 Passive Properties

As mentioned previously, the passive parameters are adjusted in order to generate appropriate passive property values according to experimentally estimated ranges (Gentet et al, 2000). Furthermore, in the case of the M2 morphology, passive parameter values in the soma and dendrites are kept the same as the passive parameter values in the M1 morphology, but the
passive parameter values in the remaining axon sections are readjusted in order to get appropriate resting membrane potential, input resistance and membrane time constant values. This adjustment is required to compensate for the axonal surface area difference and is a strategy that has been used previously (Lawrence et al, 2006). Results for passive properties are summarized in table 3.

Table 3. Passive property measurements (yellow) and passive parameter values (green) for both computational morphologies (without active properties) as compared with the experimental (yellow) and literature (green) values. Note that for M2, the parameter values listed refer to the values set for the remaining axon segments. The M2 somatic and dendritic parameter values are the same as those listed for M1.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>$R_N$ (MΩ)</th>
<th>$\tau_m$ (ms)</th>
<th>RMP (mV)</th>
<th>$C_m$ (uF/cm$^2$)</th>
<th>$R_a$ (MΩ)</th>
<th>$G_m$ (S/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>411.8</td>
<td>24.2</td>
<td>-69.7</td>
<td>0.9</td>
<td>255</td>
<td>0.000019</td>
</tr>
<tr>
<td>M2</td>
<td>414.3</td>
<td>24.2</td>
<td>-69.7</td>
<td>4 (Axon)</td>
<td>300 (Axon)</td>
<td>0.000185 (Axon)</td>
</tr>
<tr>
<td>Experimental/Literature</td>
<td>413.0</td>
<td>24.2</td>
<td>-69.7</td>
<td>0.9 ± 0.3</td>
<td>100-300</td>
<td>N/A</td>
</tr>
</tbody>
</table>

3.2.3 Active Properties

3.2.3.1 Initial Channels

Once the passive properties are modeled, we then consider several different channel types with different spatial layouts along the morphology. The following four types of channels are first considered: transient sodium current ($I_{Na,t}$), slow delayed rectifier potassium current ($I_{KdrS}$), fast delayed rectifier potassium current ($I_{KdrF}$) and A-type potassium current ($I_{K_a}$). These channel models are obtained from the OLM cell model developed in Lawrence et al, 2006. These channels are chosen to determine whether and how their densities and kinetics allowed control over basic action potential dynamics (e.g. depolarization, repolarization, ISI, stimulus response) through voltage-gated channeling. They therefore seem reasonably necessary for replicating the observed firing patterns and action potential measurements seen in IS3 cells. Additionally, preliminary data from IS3 cells (unpublished observations, Topolnik Lab) suggests that they express intrinsic, subthreshold activities similar to other hippocampal interneuron types (i.e. such
as in Chapman and Lacaille, 1999). Previous models captured this aspect with the incorporation of a persistent sodium current ($I_{Na,p}$), an $I_{Ka}$ and additive white noise ($I_{noise}$) to represent stochastic gating mechanisms (Morin et al, 2010; Sritharan and Skinner, 2012). Thus, to capture these aspects in our developing models, we include somatic $I_{Na,p}$ current and somatically injected white noise current. Altogether, these channels are the first set of channels to be investigated (i.e. in cases 1-3 – see table 2). In terms of the differences between cases (table 2), each case looks at unique distributions of channel types along the morphology of the cell. Note that all channel descriptions and equations can be found in appendix A, along with channel kinetic plots.

3.2.3.2 PSIBAuRg: Cases 1-3

Although the PSIBAuRg process is repeated for many different cases of channels, I will mainly focus on explaining the transition from cases 1-3 to 7-9 (see table 2 for more details on these cases). Generally, cases are investigated in threes where Case 1 = channels in soma only, Case 2 = channels in soma and dendrites (except $I_{Na,p}$) and Case 3 = channels in soma and dendrites (except $I_{Ka}$ and $I_{Na,p}$). Therefore the only differences between cases 1-3 and 7-9 are channel types and conductance ranges.

To begin with cases 1-3 (see figure 4), we generally see that there is a fairly large parameter space that yields models that are capable of eliciting the key features of IS3 cells (i.e. that pass the pre-processing stage). However, looking more closely at the distributions of measurements (figure 5) found in these models, we are able to notice important points where the models seem to fail. For example, the spike threshold is too low, the half-width is too large and the spike afterhyperpolarization is too large. In addition to this, the depolarization height in the membrane potential during depolarizing current injections is also too small (i.e. not just in the 50pA CIP but in the 20pA and 500pA CIPs as well). To quantify this feature, we included a measure that calculates the difference between the average membrane potential before the current injection and the average membrane potential during the current injection (see table 1 and figure 5).

In terms of the actual conductance ranges considered for each channel, these are listed in table 4. Interestingly, once channels are distributed uniformly across the dendrites, the conductance ranges mostly need to be set to lower values, usually by almost one order of magnitude. One exception to this is the persistent sodium channel, usually because this channel has such large excitatory effects, even at low conductance values (see table 4, column 3).
Case 1

Figure 4. LEFT: Top models in cases 1-3 (from top to bottom). Current injection protocol is the same as what is seen in figure 2. RIGHT: Parameter spaces for cases 1-3 (from top to bottom),
as visualized using CBDR. Each pixel represents a single model with a given set of conductance parameter values, and the heat map coloring represents that model’s distance metric. In this sense, the conductance bars on each axis of the CBDR plots therefore represent the channel conductance magnitudes for each individual pixel. Note that there are three possible conductance value possibilities for each channel (i.e. lowest, middle and highest according to the lowest and highest values listed in the plots) and the size of the bars represents the number of pixels that are covered by a given conductance value. Conductance axes are organized such that overall low conductance models are in the bottom left quadrants and overall high conductance models are in the top right quadrants. Black pixels represent models that are rejected during pre-processing and are assigned a distance metric value of 100 as a result.
Figure 5. Histograms of some of the measurements (top to bottom: average membrane potential difference at 50pA, average spike half-width, average spike threshold and average spike afterhyperpolarization) for case 1 post-processed models (left), as compared with the experimental measurements (right). The red dashed line in the experimental histograms represents the measurements of the canonical trace shown in figure 2. Note that because of limited experimental data, experimental histograms are generated from data where the only criterion is that they exhibit regular spiking regimes (i.e. current injection protocols range from 10pA to 140pA for the spiking regime). Also note that only case 1 is shown here because case 2 and case 3 show similar measurement distributions.
Table 4. Summary of the appropriate conductance ranges found using the PSIBAuRg approach for cases 1-3 and 7-9.

<table>
<thead>
<tr>
<th>Case #</th>
<th>$G_{Na,t}$ (S/cm²)</th>
<th>$G_{Na,p}$ (S/cm²)</th>
<th>$G_{K_d}$ (S/cm²)</th>
<th>$G_{K_{drf}}$ (S/cm²)</th>
<th>$G_{K_{drs}}$ (S/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.16-0.22</td>
<td>0.0001-0.0003</td>
<td>0.05-0.15</td>
<td>0.03-0.11</td>
<td>0.03-0.09</td>
</tr>
<tr>
<td>2</td>
<td>0.02-0.04</td>
<td>0.0001-0.0003</td>
<td>0.0025-0.0075</td>
<td>0.002-0.01</td>
<td>0.006-0.012</td>
</tr>
<tr>
<td>3</td>
<td>0.02-0.04</td>
<td>0.0001-0.0003</td>
<td>0.0025-0.0075</td>
<td>0.002-0.01</td>
<td>0.006-0.012</td>
</tr>
<tr>
<td>7</td>
<td>0.2-0.25</td>
<td>0.00005-0.00015</td>
<td>0.15-0.25</td>
<td>0.95-1.05</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.04-0.06</td>
<td>0.0002-0.0004</td>
<td>0.06-0.1</td>
<td>0.1-0.16</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.04-0.05</td>
<td>0.00005-0.00015</td>
<td>0.3-0.32</td>
<td>0.1-0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

3.2.3.3 Delayed Rectifier Potassium Subunit Analysis Using Allen Mouse Brain Atlas

Although there does not seem to be a clear and straightforward way of increasing the depolarization height in the membrane potential during depolarizing currents, we find (through hand-tuning) that by using faster delayed rectifier potassium kinetics, we can improve the models by decreasing the spike half-width and afterhyperpolarization. This in turn, allows for conductance balances that increase the depolarization height in the membrane potential during depolarizing currents. In terms of using faster delayed rectifier potassium kinetics, one option is to remove slow delayed rectifier channels from the model, and the other is to use faster time constants in the fast delayed rectifier channel model.

As mentioned previously, for slow delayed rectifier potassium channel subunit composition, it is generally known that Kv2.1 or Kv2.2 combine with Kv5.1, Kv6.4 or Kv9.1-Kv9.3 (Lien et al., 2002; Coetzee et al., 1999). Fast delayed rectifier potassium channels on the other hand, are generally known to be composed of Kv3.1 and/or Kv3.2 subunits, of which Kv3.1 composition seems to yield faster time constants (Lien et al., 2002; Hernández-Pineda et al., 1999). To get an idea of expression of these subunits in the Radiatum layer of the hippocampus (i.e. the layer where IS3 cell bodies and the large majority of its dendrites are located), we look at in situ
hybridization data from mouse brain slices using the Allen Mouse Brain Atlas (Lein et al., 2007; See http://help.brain-map.org/display/mousebrain for full documentation).

For the slow delayed rectifier subunits, it appears as though there is very little expression if any in the Radiatum or Lacunosum-Moleculare layers (see figure 6 for example of Kv2.1 expression), which further suggests that slow delayed rectifier channels might not be prominent in IS3 cell bodies or dendrites. For fast delayed rectifier subunits, it appears as though Kv3.1 subunit expression is much more prominent in the Radiatum layer than Kv3.2 subunits (see figure 7 for comparison), which suggests that faster time constants might be a feature seen in IS3 cell fast delayed rectifier potassium channels. For full channel subunit analysis, see Appendix B.

Figure 6. In situ hybridization data showing genetic expression of KCNB1 (i.e. the gene that encodes Kv2.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Highest genetic expression of KCNB1 is seen in the sp layer with some expression in so, whereas there is no observable expression in slm or sr layers. Image credit: Allen Institute for Brain Science. URL:
http://mouse.brain-map.org/experiment/siv?id=74047856&imageId=74010263&initImage=ish&coordSystem=pixel&x=8135.545846233195&y=3869.6160130760577&z=3
Figure 7. In situ hybridization data showing genetic expression of KCNC1 (top) and KCNC2 (bottom) (i.e. the genes that encode Kv3.1 and Kv3.2 subunits, respectively) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). KCNC1 seems to be highly expressed mostly in sp and sr, whereas KCNC2 expression seems to be more localized in sp, so and slm (highest to lowest,
respectively) with some expression in distal sr (i.e. close to slm border). Image credit: Allen Institute for Brain Science.

**KCNC1 URL:**
http://mouse.brain-map.org/experiment/siv?id=72108825&imageld=71997475&initImage=ish&coor

dSystem=pixel\&x=8162.67115555686\&y=3053.8295182756137\&z=3

**KCNC2 URL:**
http://mouse.brain-map.org/experiment/siv?id=75147765&imageld=75165793&initImage=ish&coor

dSystem=pixel\&x=8185.54302320509\&y=3644.8008502721314\&z=3

### 3.2.3.4 PSIBAuRg: Cases 7-9

With the results from cases 1-3 and the observations from Allen Mouse Brain Atlas in mind, we then repeat the PSIBAuRg approach but without slow delayed rectifier potassium channel and with a more generic fast delayed rectifier channel containing faster delayed rectifier potassium channel kinetics (i.e. obtained from Saraga *et al*, 2003 – see Appendix A for details). Once again, we look at a case with only somatic channels and two cases with channels uniformly distributed across the soma and dendrites (i.e. one without $I_{Na,p}$ in the dendrites and one without $I_{K,a}$ and $I_{Na,p}$ in the dendrites). These three additional cases (i.e. cases 7-9) are once again outlined in table 2.

The results from cases 7-9 are displayed in figures 8 and 9. For all three cases, measurement histograms generated from the models in each database show more similar distributions to the experimental measurement histograms when compared with cases 1-3 (figure 9). For these cases we see an increase in the depolarization height of the membrane potential during depolarizing currents. However it is still not quite as large as those seen in the experimental recordings. This is reflected in the histograms in figure 9 for the average membrane potential differences (top) and average spike thresholds (third from top), where there is minimal and no overlap with the experimental histograms, respectively. On the other hand, we do see large improvements in other measurements such as the spike half-widths and the spike afterhyperpolarizations (figure 9). Importantly, this allows us to distinguish that the changes made in cases 7-9 from cases 1-3 lead to measured improvements in the models.
Figure 8. LEFT: Top models in cases 7-9 (from top to bottom). Current injection protocol is the same as what is seen in figure 2. RIGHT: Parameter spaces for cases 7-9 (from top to bottom), as visualized using CBDR. Each pixel represents a single model with a given set of conductance
parameter values, and the heat map coloring represents that model’s distance metric. In this sense, the conductance bars on each axis of the CBDR plots therefore represent the channel conductance magnitudes for each individual pixel. Note that there are three possible conductance value possibilities for each channel (i.e. lowest, middle and highest according to the lowest and highest values listed in the plots) and the size of the bars represents the number of pixels that are covered by a given conductance value. Conductance axes are organized such that overall low conductance models are in the bottom left quadrants and overall high conductance models are in the top right quadrants. Black pixels represent models that are rejected during pre-processing and are assigned a distance metric value of 100 as a result.
Figure 9. Histograms of some of the measurements (top to bottom: average membrane potential difference at 50pA, average spike half-width, average spike threshold and average spike afterhyperpolarization) for case 7 post-processed models (left), as compared with the experimental measurements (right). The red dashed line in the experimental histograms represents the measurements of the canonical trace shown in figure 2. Note that because of limited experimental data, experimental histograms are generated from data where the only criterion is that they exhibit regular spiking regimes (i.e. current injection protocols range from 10pA to 140pA for the spiking regime). Also note that only case 7 is shown here because case 8 and case 9 show mostly similar measurement distributions.

In terms of distinguishing which of the cases is better between cases 7, 8 and 9, we look at several additional factors. In figure 10 we look at the first spike time distributions in each database. Experimental recordings indicate that during regular spiking regimes, the first spike time of the current injection is usually early on (i.e. between 0 and 40ms). Although case 7 ranges from spikes starting as early as 40ms and ranges to about 120ms, cases 8 and 9 range...
from 50ms to 350ms and 200ms, respectively. This indicates that spike onset is often too late in all the models, but that case 7 seems to perform best. Another factor that distinguishes case 7 from cases 8 and 9 is the presence of spike amplitude decreases during the regular spiking regime (i.e. at 50pA). Whereas the models generated in cases 8 and 9 have spike amplitudes that increase during regular spiking, case 7 has the opposite: spike amplitudes that start off large and then decrease over the course of the current injection. This is observed in the top model for case 7 in figure 8, as compared with the “canonical” experimental recording in figure 2. As mentioned previously, decreasing amplitudes is a common feature observed in the experimental data, and it is therefore an important feature to replicate in our models. In cases 7–9, case 7 is the only case that captures this IS3 feature.

Figure 10. First spike time histograms generated from 50pA simulations (case 7 – top left, case 8 – top middle, case 9 – top right) and experimental spiking regime recordings (bottom). The red
dashed line in the experimental histogram represents the measurements of the canonical trace shown in figure 2. Note that because of limited experimental data, experimental histograms are generated from data where the only criterion is that they exhibit regular spiking regimes (i.e. current injection protocols range from 10pA to 140pA for the spiking regime).

Once again, the conductance value ranges are listed in table 4. Like cases 1-3, distributing the channels uniformly across the dendrites requires an overall decrease in conductance values (i.e. with the exception of persistent sodium). An additional exception to this is found in case 9, however, where the only scenario where appropriate models are generated requires a large conductance of somatic A-type potassium (i.e. almost twice as large as in case 7). For the most part, the conductance ranges are similar between cases 1-3 and cases 7-8, with the exception of the potassium channels, which generally have higher conductance values in cases 7-8, likely due to the removal of the slow delayed rectifier channel.

3.2.3.5 Pairwise Channel Co-regulations in Case 7

Since case 7 appears to be the most successful case for generating appropriate models, we additionally look at pairwise channel co-regulations in this case (figure 11) using terminology similar to what is used in Sekulić et al, 2014. In this sense, pairwise conductance values are classified as either, 1) no clear interaction, 2) local peak conductance preference or 3) co-regulation. For the most part, every pair of channels appears to have local maxima in their histograms, but these are most pronounced in every pair that includes A-type potassium channels. In this sense, channel co-regulation is observed in every pairwise combination that includes A-type potassium, and local peaks are observed in the remaining three pairwise combinations. Because every pair of channels seems to have some co-regulation, this further confirms that each channel in case 7 is playing a significant role in the generation of appropriate models based on our criteria.
Figure 11. Channel co-regulations found in the case 7 model post-processed database for all possible channel conductance pairs plotted against number of appropriate models (vertical axis). In all cases there appears to be local maxima where the number of appropriate models is maximized, but the maxima are more extreme in the co-regulation plots involving A-type potassium (i.e. plots on left side).
3.2.3.6  Dendritic Spike Propagation in Case 7 and 8 Top Models

Based on this approach so far, it seems as though case 7 is a likely scenario for IS3 cell channel types and distributions. However this suggests that channels in IS3 cells are restricted to the soma and that uniform dendritic channels are not a suitable component for normal IS3 electrophysiological activity. To further test this, we simulate dendritic recordings along the dendrites of two of our models: the top model from case 7 and the top model from case 8 (see table 5 for conductance values). Specifically, we use a similar methodology to what is used experimentally where we inject somatic current with an amplitude of 800 pA for a duration of 2 ms (figure 12). We then look at action potential amplitude decay with distance from soma and compare it to calcium signal amplitude decay seen in experimental recordings. As can be observed in figure 12, although case 7 dendritic action potentials decay, they are still observable at large distances from the soma (i.e. small ~40 mV amplitude spikes are still observed at 200 µm from the soma). In fact the proportion of action potential amplitude decay in case 7 seems comparable to the proportion of calcium signal decay, with the exception of a lack of decay seen in the first 50 µm of dendrite in experimental recordings. Case 8 on the other hand does not decay at all, regardless of distance from soma, which seems to be expected because of the high density of active properties distributed uniformly across the dendrites. Altogether, these results seem to suggest active properties in at least the first 50 µm of dendrite.
Figure 12. Top left: Morphological M2 model showing physical size of the cell model in micrometers. Top right: Schematic view of the M2 model with the dend[3] section highlighted in red. Because this section is divided into multiple compartments, we can measure electrophysiological differences between compartments. The total length of this section is 202.88533 µm, as measured using the distance() function in NEURON. Bottom left: Action potential amplitude deterioration along dend[3] in the case 7 top model following a somatic 800pA current injection for 2 ms ($G_{Na,t} Soma = 0.25$ S/cm$^2$; $G_{Na,p} Soma = 0.0001$ S/cm$^2$; $G_{K_a} Soma = 0.15$ S/cm$^2$; $G_{K_{drf}} Soma = 1$ S/cm$^2$). Bottom right: Action potential amplitude deterioration along dend[3] in the case 8 top model following a somatic 800pA current injection for 2 ms ($G_{Na,t} Soma/Dendrites = 0.06$ S/cm$^2$; $G_{Na,p} Soma = 0.0002$ S/cm$^2$; $G_{K_a} Soma/Dendrites = 0.1$ S/cm$^2$; $G_{K_{drf}} Soma/Dendrites = 0.1$ S/cm$^2$).
Table 5. Summary of conductance values in the top models from cases 7 and 8 as well as model 8* and 9*.

<table>
<thead>
<tr>
<th>Model</th>
<th>Distribution</th>
<th>$G_{Na,t}$ (S/cm$^2$)</th>
<th>$G_{Na,p}$ (S/cm$^2$)</th>
<th>$G_{Ka}$ (S/cm$^2$)</th>
<th>$G_{Kdr}$ (S/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 7 Top</td>
<td>Soma only</td>
<td>0.25</td>
<td>0.0001</td>
<td>0.15</td>
<td>1</td>
</tr>
<tr>
<td>Model</td>
<td>Uniform across soma and dendrites</td>
<td>0.06</td>
<td>0.0002</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Case 8*</td>
<td>Uniform across soma and first 50 µm of dendrites</td>
<td>0.075</td>
<td>0.0001</td>
<td>0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>Case 9*</td>
<td>Uniform across soma and first 50 µm of dendrites</td>
<td>0.07</td>
<td>0.0001</td>
<td>0.05</td>
<td>0.3</td>
</tr>
</tbody>
</table>

3.2.3.7 Channels in Proximal Dendrites: Case 8* and 9*

In order to test whether dendritic channels up to 50 µm along the dendrites has an effect of reducing action potential amplitude decay for recordings along the first 50 µm of dendrites, we use NEURON’s SubsetDomainIterator function in CellBuilder to specify channel conductance variation along the dendrites of the IS3 model. Specifically, we use a Boltzmann function to describe the relationship between channel conductance and distance from soma:

$$f(p) = \frac{G}{1 + \exp(k \times (d - p))}$$

Where $f(p)$ is the distance-dependent conductance, $p$ is the distance along the dendrite, $G$ is the desired conductance value in the first 50 µm of dendrite, $k$ is a value of 10 in order to ensure a sharp decrease in conductance at 50 µm and $d$ is a value of 55 in order to ensure that the decrease in conductance occurs at around 50 µm. See figure 13 (top) for a plot of this relationship if $G$ were a value of 1 S/cm$^2$. In this plot we see that the “desired” conductance value is very predictive of the distance-dependent conductance, $f(p)$, that results from the Boltzmann function.

In figure 13, we see the results from a hand-tuned case 8* model (i.e. channels in soma and dendrites except persistent sodium) and a hand-tuned case 9* model (i.e. channels in soma
and dendrites except persistent sodium and A-type potassium) with uniform channels in the first 50 µm of the dendrites (see table 5 for conductance values). From the traces for both these models, we see that they appear to be very similar in quality to the case 7 models, which shows that a scenario with active properties in the first 50 µm of dendrites is capable of generating appropriate somatic recordings. Also, using the same dendritic recording procedure (i.e. a 800 pA, 2 ms duration somatic current injection), we see that dendritic spike amplitude decay is attenuated in the first 50 µm of dendrites in these two scenarios, unlike the continuous spike amplitude decay seen in case 7.
Figure 13. Top: Plot of Boltzmann function where $G$ is a value of $1 \text{ S/cm}^2$, $k$ is a value of 10 and $d$ is a value of 55. Resultant plot shows a value of $1 \text{ S/cm}^2$ along the dendrite until $50 \mu m$ where the conductance drops sharply to $0 \text{ S/cm}^2$. Middle left: Case 8* hand-tuned model with channels that are uniform from the soma until the first $50 \mu m$ of the dendrites using the Boltzmann function. Conductance values are the following: $G_{Na,t}^{Soma/Dendrites} = 0.075 \text{ S/cm}^2$, $G_{Na,p}^{Soma} = 0.0001 \text{ S/cm}^2$, $G_{Kdrf}^{Soma/Dendrites} = 0.3 \text{ S/cm}^2$, $G_{K_a}^{Soma/Dendrites} = 0.05 \text{ S/cm}^2$. Middle right: Case 9* hand-tuned model with channels that are uniform from the soma until the first $50 \mu m$ of the dendrites using the Boltzmann function. Conductance values are the following: $G_{Na,t}^{Soma/Dendrites} = 0.07 \text{ S/cm}^2$, $G_{Na,p}^{Soma} = 0.0001 \text{ S/cm}^2$, $G_{Kdrf}^{Soma/Dendrites} = 0.3 \text{ S/cm}^2$, $G_{K_a}^{Soma} = 0.05 \text{ S/cm}^2$. Note that the current injection protocol is the same as what is seen in figure 2. Bottom left: Action potential amplitude deterioration along dend[3] in the above case 8* hand-tuned model following a somatic $800 \mu A$ current injection for 2 ms. Bottom right: Action potential amplitude deterioration along dend[3] in the above case 9* hand-tuned model following a somatic $800 \mu A$ current injection for 2 ms.

3.2.4 Usages

3.2.4.1 Irregular Firing Analysis in Top Models

As mentioned previously, experimental IS3 cell recordings have also been shown to elicit irregular spiking activity (i.e. ISI CVs greater than 1) when depolarized near firing threshold. To demonstrate that the models generated here can elicit irregular spiking when held near threshold, we test the case 7 and 8 top models as well as the case 8* and 9* models across a range of near threshold current injections ($0.02nA-0.05nA$) as well as across a range of somatic noise magnitudes ($0.01nA-0.1nA$). In all cases, the models are capable of eliciting ISI CVs greater than 1.
1, though only the case 7 top model as well as the case 8* and 9* models consistently generate ISI CVs greater than 1 when held near threshold (figure 14). On the other hand, the case 8 top model more often generates ISI CVs greater than 0.5 and only sometimes peaks above 1 in some instances. Additionally, the case 7 top model, the case 8* model and the case 9* model had firing thresholds near 0.02 nA whereas the case 8 top model had firing threshold near 0.04 nA. Finally, ISI CV peaks (see traces in figure 14) usually occur during mid-range noise magnitudes (i.e. peaks at ~\(I_{\text{noise St. Dev.}} = 0.05\) nA) with the exception of the case 8 top model, where ISI CV seems to increase with noise magnitude (i.e. peaks at 0.09 nA).
3.2.4.2 Firing Reliability Analysis in Top Models

So far we have generated a database of appropriate models in case 7 and have shown that a uniform distribution of channels in the first 50 µm of the dendrites can also generate appropriate models. Furthermore, these latter models (i.e. case 8* and 9*) seem to bear more resemblance to the experimental IS3 dendritic calcium imaging data. In addition, we hypothesized that dendritic VGCs are important for reliable spiking in IS3 cells. To test this, we model single presynaptic spikes at different locations along the dendritic arbors of the case 7 top model, the case 8 top...
model, the case 8* model and the case 9* model, in order to find the minimum weights necessary
to evoke spikes at the soma. The results from these tests are summarized in figure 15.

Interestingly, there are typically three distinct dendritic points where threshold weight rises
sharply in the graphs in figure 15. Each one represents a main dendritic tree (see the subset
morphologies in figure 15). Tree 1, for example, has less branching and longer sections than the
other dendritic trees and can generate somatic spikes with below 0.5 uS weights up until roughly
325-375 µm. Tree 2A, which was the second most reliable subtree (i.e. below 0.5 uS threshold
weights up until roughly 200-250 µm), also has less branching and longer sections than tree 2B,
which was the least reliable subtree (i.e. below 0.5 uS threshold weights up until roughly 150-
200 µm).

In the case 7 top model we see that spike generation is reliable in the first 100 to 325 µm of the
dendritic arbor, depending on the dendritic branch being analyzed. By reliable we mean that the
threshold weight to elicit a somatic spike is below 0.5 uS (i.e. approximately where the increase
in threshold weight usually occurs). In case 8*, we see very similar results, which suggests that
the dendritic channel distribution and balances (i.e. I_{Na,t}, I_{Kdrf} and I_{Ka}) does not improve spike
reliability from a passive scenario. In case 9*, however, we see that reliability extends by about
50 µm in all of the branches, indicating that by not including A-type potassium in the dendrites,
spike generation is facilitated.

Finally, in the case 8 top model we see that spiking is reliable (i.e. < 0.5 uS threshold weight)
regardless of the location of the synapse. Interestingly, the threshold weight seems to decrease
with distance from the soma, suggesting that uniform dendritic active properties across the entire
dendritic arbor can amplify distal inputs over proximal inputs. However, since the threshold
weight is fairly small across all the synaptic locations, it seems unlikely that any significant
preference for distal inputs over proximal inputs would be observed.
Case 7 Top Model

Distance dependent synaptic weight thresholds

Case 8 Top Model

Distance from soma (um)
Case 8* Model

Distance dependent synaptic weight thresholds

Case 9* Model

Distance dependent synaptic weight thresholds
Figure 15. Synaptic weight threshold (μS) necessary to evoke a somatic spike in response to a single presynaptic spike applied incrementally at different points along the dendritic arbor (top to bottom: case 7 top model, case 8 top model, case 8*, case 9*). Note that each divergent line represents measurements from a branch diverging from the main dendritic arbor and that the y-axis in the case 8 top model (top right) is times $10^{-3}$. The subset morphology plots indicate the divergent spike reliability paths measured from each of the main subtrees of the dendritic arbor (i.e. they highlight reliability differences due to morphological differences). The dotted red lines represent the border threshold weight (i.e. 0.5 μS) at which synapses at further locations from the soma are no longer considered to reliably elicit spikes (i.e. past this distance from the soma, the threshold weights tend to become much larger).

3.2.4.3 Spike Time Precision in Top Models

In this section, we test how much the presence of irregular spiking in our models affects the spike timing, for given a synaptic input. As mentioned previously, while stochastic gating (i.e. an important component for irregular firing) seems to generally be detrimental for spike time precision (Schneidman et al, 1998; Schreiber et al, 2004; Ozer et al, 2009) the inclusion of channels that allow subthreshold spectral regimes to occur may be beneficial for spike time precision (Schaefer et al, 2006; Morin et al, 2010; Sritharan and Skinner, 2012). As demonstrated above, irregular firing is maximized in our models during minimal near-threshold activation (i.e. figure 14), so for this reason, we run multiple short simulations of our models (i.e. case 7 and 8 top models and case 8* and 9* models) using near-threshold somatic synaptic inputs and a range of Gaussian noise magnitudes. By analyzing how closely timed each model’s spikes are between repeats of the simulation, we can approximate (i.e. by looking at the precision of spike times) how likely each model is to cause synaptic summation in OLM cells. For example, since it is known that an IS3 frequency of 100Hz is necessary for synaptic summation to occur in OLM cells (Tyan et al, 2014), we know that spikes from two separate IS3 cells need to arrive within 20ms (i.e. 2 spikes / 0.02 s = 100 Hz) of each other in order for this to happen.

From figure 16 we see that the timing of spikes does not vary a lot (i.e. as quantified by the standard deviation), even when the models are near threshold. As seen in the scatter/histogram plots of figure 16, the higher standard deviations seen when the models are near a synaptic weight threshold occurs because the spike latency is increased and the models sometimes fail to
spike in these regimes due to the presence of noise. This would have an effect of increasing the standard deviation because the number of spikes \((N)\) would be smaller. Additionally, we see in the case 7 and 8 top models that spike time precision decreases with noise magnitudes. However, even in these cases, the standard deviations are quite small throughout (i.e. <10ms).

These results indicate that once the synaptic weight is large enough to elicit a spike, the spike times are fairly consistent. As mentioned, from Tyan et al, 2014 we know that an IS3 spike rate needs to be around 100Hz or higher to get synaptic summation in OLM cells. Here we show that once multiple copies of the same IS3 model are activated, they always display spike delay time scales that would allow for greater than 100Hz activation in OLM cells (assuming that there are 6-7 IS3 cells for every one OLM cell as postulated in Tyan et al, 2014). In other words, if the delay between the activation of two different IS3 cells is 10ms (i.e. larger than any of the standard deviations seen in our results), then the downstream OLM cell would receive input at a rate of \((2 \text{ spikes} / 0.01 \text{ seconds}) = 200\text{Hz}\), which is already more than sufficient for synaptic summation. These results therefore predict that despite irregular firing patterns, IS3 cells are capable of eliciting spike times that are precise enough to control OLM cell activity.

Case 7 Top Model

Case 8 Top Model
Figure 16. Precision of spike times in case 7 and 8 top models as well as case 8* and 9* models across a range of synaptic weights (note the scale is $10^{-3}$) and noise magnitudes (top 3D plots). Models are stimulated by one presynaptic event ten times and resulting spike times are recorded. Note that empty spaces represent instances where the model spikes less than twice out of ten times. Bottom plots are a combination of scatter and histogram plots where each color/line type
represents spike times from one of the simulations in the above 3D plots. Y axis histogram plots show that there are larger spike counts (N) in simulations that have better spike time precision (i.e. less spike failures). X axis histogram plots demonstrate that simulations with smaller spike latencies tended to fire more precisely (i.e. lower standard deviations). Note that a kernel smoothing window was used in the histogram plots.
4 Discussion

4.1 Summary

In this research thesis, we develop an efficient approach to estimating the active properties of single neurons that utilizes electrophysiological data (section 3.1.1) and dendritic calcium imaging (section 3.1.2) to make experimentally constrained predictions. Using this approach, we generate databases of models that mimic the various features seen in IS3 cell experimental recordings (section 3.2.3). From these models we obtain estimates of the VGC types, densities and distributions that may be found in IS3 cells.

Furthermore, when tested, we find that all the most highly ranked models are capable of generating irregular firing near threshold (section 3.2.4.1), as is seen in IS3 cells. Despite this characteristic, we also demonstrate that, when activated, it is possible for our IS3 cell models to control the activity of an OLM cell through synaptic summation of precisely timed IS3 spikes (section 3.2.4.3). When analyzing the minimal weights necessary to elicit a spike along the dendritic arbor of our models (section 3.2.4.2), we find that these weights are minimized when the models possess uniform distributions of channels along the dendrites. Interestingly, for the models with non-uniform VGC distributions along the dendrites (i.e. VGCs in the first 50 µm of dendrites), the synaptic weights necessary to elicit a somatic spike are minimized when A-type potassium channels are restricted to the soma. In terms of how well each model replicates IS3 cell features, we find that uniform distributions of channels along the dendrites do not acceptably generate the electrophysiological regimes of IS3 cells. On the other hand, passive dendritic cases do not allow the dendritic action potential propagation that is extrapolated from IS3 dendritic calcium imaging data. For these reasons we assume that the non-uniform dendritic VGC distribution models offer the most likely scenario for IS3 cell active properties.

Functionally, these non-uniform dendritic VGC distributions may serve several functions. For example, having large densities of VGCs in the proximal dendrites of IS3 cells (i.e. in CA1 SR) may serve to amplify inputs from CA3. Since it is known that OLM cells are silenced during SWRs (Katona et al, 2014), it is thought that IS3 cells might be responsible for this by receiving excitatory inputs from CA3 during these events. VGC densities in the proximal dendrites of IS3 cells might serve to efficiently facilitate the transient SWR recruitment of IS3 cells. In terms of
input onto the passive distal dendrites (i.e. in SLM) of IS3 cells in these models, single presynaptic events are much less likely to induce spikes (as is seen in figure 15). However, given precisely timed activation from multiple inputs, synaptic summation in IS3 cells could potentially still lead to somatic spikes. As stated in section 1.3.3, IS3 cell distal dendrites may also be receiving inputs from entorhinal cortex via the temporoammonic pathway (Francavilla et al, 2015). Given that it would be necessary for the IS3 cell model’s passive distal dendrites to receive precisely timed inputs in order for it to spike, synchronous spiking from entorhinal cortex inputs onto IS3 cells may enable the recruitment of IS3 cells.

4.2 Predictions

Given the present correspondence with data, these models already make predictions about somatic channel types, distributions and balances found in IS3 cells. Specifically, these results predict several major IS3 characteristics:

1) IS3 cells do not possess high densities of slow delayed rectifier channels, if any
2) IS3 cells are likely to highly express Kv3.1 fast delayed rectifier channel subunits
3) VGCs in IS3 cells are likely distributed across the soma and proximal dendrites
4) The presence of moderate stochastic gating (i.e. $I_{\text{noise}}$ magnitude) allows irregular firing in IS3 cells when activated near firing threshold
5) The maximal distance from soma at which a threshold weight of less than 0.5 uS can elicit a spike is maximized when A-type potassium channels are restricted to the soma
6) The maximal distance from soma at which a threshold weight of less than 0.5 uS can elicit a spike is also maximized when the dendrite sections are long with minimal branching
7) Despite irregular spiking, minimally activated IS3 cells are capable of eliciting spike times that are precise enough to control OLM cell activity
8) Conductance values are likely high for $I_{\text{Kdrf}}$, moderate for $I_{\text{Ka}}$ and $I_{\text{Na,t}}$, and low for $I_{\text{Na,p}}$ relative to each other
9) Although $I_h$, $I_{CaT}$ and $I_{CaL}$ are likely present, they may only be playing subtle roles in IS3 cells (for explanation see “Limitations” section)

4.3 Limitations

The major morphological limitation is whether or not axonal branches are important in this scenario (M1 vs. M2 morphology). From one perspective, including it is computationally expensive and requires additional assumptions regarding axonal biophysical properties. From another perspective, excluding it can limit the uses of the model in future projects. Also it seems clear that a large proportion of IS3 surface area is axonal arborizations (i.e. 65% in the case of the first cell), which means a fairly large loss in surface area once axonal branches are removed. Importantly though, once the passive parameters are optimized in both morphologies, the inclusion of somatic channels seems to have similar effects on the action potential measurements in both the M1 and M2 morphologies.

As mentioned, the model is essentially being used to make predictions about the intrinsic properties of IS3 cells, and therefore relies on certain assumptions. A previous model of a basket cell made these predictions by relying on pre-described kinetic equations of ion channels for fast-spiking hippocampal interneurons (Saraga et al, 2006). Another previous model, of an OLM cell, relied more so on experimentally determined channel kinetics (Saraga et al, 2003; Lawrence et al, 2006). In the case of this IS3 model, though certain active properties have been characterized, it is not yet explicitly known what types of channels are actually present in IS3 cells, and therefore the channels chosen so far are based on assumptions that are made in order to fit the model with observed firing dynamics. However, this is, in essence, the purpose of this project: to make predictions regarding the channel types and distributions in IS3 cells that allow it to generate its specific membrane potential dynamics and firing patterns.

An additional limitation with this project is that, because hand tuning is a major component, it is sometimes difficult to gauge appropriate magnitudes for the parameter values, which can end up being off by several orders of magnitude. We address this limitation by adjusting the conductance ranges to their optimal parameter spaces, as visualized in CBDR plots (figure 4 and 8, right), where the maximal number of appropriate models is generated.
Also, since the model is minimalistic in regards to only having components that are shown to improve the model, several channels that are likely to be present in IS3 cells are purposely not included. Namely, these channels are T-type calcium ($I_{CaT}$), L-type calcium ($I_{CaL}$) and hyperpolarization-activated cyclic nucleotide-gated (HCN; $I_h$) channels. For one, $I_{CaT}$ and $I_h$ seem to be present due to observations of channel-specific effects on the experimental voltage recordings (i.e. during spiking and hyperpolarization, respectively). Secondly, some preliminary calcium blocking experiments seem to show that $I_{CaL}$ is present, but in small amounts.

Additionally, a previous study has also indicated that calretinin-positive cells in the CA1 area of the hippocampus express L-type calcium subunits in small proportions, as well as T-type, N-type, R-type and P-type calcium channels in larger proportions (Vinet and Sík, 2006). In general, we did run PSIBAuRg simulations with some of these channels (i.e. $I_{CaT}$ and $I_{CaL}$ – see figure D1 and tables D1-D2 in appendix D) as well as hand tuning simulations with all three, but these channels did not markedly improve the model’s measurements, in regards to the experimental trace measurements. In other words, model quality is usually maximized when conductance values for these channels are very small, regardless of the channel distribution or assortment of channel types. Note that these three channel models are all obtained from a previous OLM cell model (Lawrence et al, 2006).

It is also important to mention the limitations of using the Allen Mouse Brain Atlas to gauge the presence of certain channel types in IS3 cells. For one, having genetic expression of channel subunits in a certain hippocampal region does not necessarily implicate that that channel will be present in all cell types in that region. In fact, it seems likely that expression of a certain channel type will only reflect expression in a subset of cell types. Also, having no expression of a channel subunit might not actually reflect whether or not the channel is present but could also be due to other factors such as mouse development and methodology. Because we are limited by the number of mice (i.e. only one mouse brain was looked at for each gene) this also does not factor in variability across mice. As well, tools are not available for quantifying genetic expression across many tissue samples of SR, nor are they spatially precise enough to quantify genetic expression in a single tissue sample of SR. For these reasons, observations of genetic expression of channel subunits in the Allen Mouse Brain Atlas are mostly just taken as additional suggestive reasoning for including, removing or changing the kinetics of channels in the model (i.e. not as conclusive evidence).
It is also worth mentioning that many assumptions are being made when comparing dendritic calcium signal amplitudes (evoked by backpropagating action potentials) with dendritic action potential amplitudes, due to the many unknown processes that could be occurring in IS3 dendrites. Despite this however, for the purposes of this analysis, we have assumed that there is a relation in at least the proximal dendrites (~50 µm). Regardless, we were curious regarding whether or not dendritic calcium channels (i.e. such as L-type or T-type calcium channels) were playing large roles in propagating action potentials. To test this, we re-designed the simulations shown in figure 12 to include dendritic calcium channels ($G_{CaT} = 0.0001$ S/cm$^2$ and $G_{CaL} = 0.0001$ S/cm$^2$) in the top models from cases 7 and 8. In these simulations we did not observe any noticeable changes in the spike amplitude decay or lack of spike amplitude decay for case 7 and 8, respectively. This seems to suggest that these calcium channels alone are not sufficient to alter the propagation of action potentials. However this is without considering other calcium related channels that are likely to be present in IS3 cells. Importantly, as mentioned in the introduction, previous studies have shown that calcium imaging can be highly predictive of action potential amplitude in other hippocampal cell types (Spruston et al, 1995; Golding et al, 2001; Martina et al, 2000; Topolnik et al, 2009; Hu et al, 2010; Camiré and Topolnik, 2014).

Finally, in our IS3 model we incorporate a very simple model of stochastic gating noise using Gaussian white noise. Relative to more realistic models of stochastic gating (e.g. Fox, 1997; Dorval, 2006; Goldwyn et al, 2011), our noise model is not voltage-dependent, it is not dependent on ion channel activity and it possesses a flat frequency distribution (i.e. with no spectral preferences). Despite this, we know from previous work that Gaussian white noise, in combination with specific VGC types, is sufficient to elicit both irregular firing (Stiefel et al, 2013) as well as subthreshold spectral properties (Morin et al, 2010; Sritharan and Skinner, 2012; Yoshida et al, 2011). Also, since we do not know the specific biological details of stochastic gating in IS3 cells, it is reasonable to use a simplified model. This also happens to be a more computationally efficient approach than using a more realistic model for stochastic gating. With this in mind, it is also worth noting that our results concerning irregular firing and spike time precision are reliant on this simple model of stochastic gating noise. Since we are interested here in simply whether stochastic gating plays a role in generating IS3 specific electrophysiological activities (and from our results we see that it has the potential to play a large role), and not the specific mechanisms of stochastic gating in IS3 cells, we make the assumption...
that a simple model for stochastic gating is an adequate approximation for the tests that we have simulated.

### 4.4 Future Work

In terms of future considerations, it could be possible to use our top developed models as base, reference models for larger-scale model database approaches to help overcome some of the limitations regarding searching for appropriate parameter values (Sekulić et al., 2014).

Ultimately, this model can be used to help predict required inputs to IS3 cells for them to functionally contribute to network oscillations such as theta. Although IS3 cells have been shown to exhibit both irregular and regular adaptive firing, the conditions required for either of these occurrences is unknown. To further investigate the conditions necessary for these firing patterns to occur, it will be necessary for experimentalists to investigate the types of excitatory and inhibitory inputs that could possibly be controlling IS3 firing. As mentioned previously, several possible excitatory pathways include excitatory input through Schaffer collateral-associated cells and perforant path-associated cells (i.e. temporoammonic pathway) (see figure 17). In this sense, by modeling different layer specific excitatory synaptic inputs to the developed IS3 model, we can get a sense of what types of inputs can drive firing patterns similar to what is observed in actual IS3 cells during electrophysiological recordings.

As well, once the properties of IS3 cells are more defined and understood, it might be possible to consider reduced versions of this model (e.g. one that only includes essential IS3 firing properties) for use in network models involving IS3 cells synapsing onto OLM cells.
Figure 17. (Left taken from Tyan et al, 2014 and right taken from Vida, 2010) Excitatory inputs from Schaffer collateral-associated cells occur in the stratum Radiatum or stratum Oriens/Alveus layers and therefore might form perisomatic synapses onto IS3 cells. Excitatory inputs from perforant path-associated cells occur in the stratum Lacunosum-Moleculare layer and therefore might synapse onto IS3 dendritic branches.
Abbreviations

Cell Types:
IS(1-3): Interneuron Specific 1-3
OLM: Oriens Lacunosum-Moleculare

Neurochemical Markers:
CR: Calretinin
CB: Calbindin
CCK: Cholecystokinin
SOM: Somatostatin
PV: Parvalbumin
VIP: Vasoactive Intestinal Polypeptide
mGluR1a: Type 1a Metabotropic Glutamate Receptor

Neuroanatomy:
CA1, CA2 and CA3: Cornu Ammonis 1-3
SO/A: Stratum Oriens/Alveus
SP: Stratum Pyramidal
SR: Stratum Radiatum
SLM: Stratum Lacunosum-Moleculare
CNS: Central Nervous System

Miscellaneous:
VGC: Voltage Gated-Channel
SWR: Sharp Wave Associated Ripple
LTP: Long-Term Potentiation
PSIBAuRg: Parameter Search Investigation By Automation and Retrofit Hand-Tuning
CBDR: Clutter Based Dimensional Reordering
CIP: Current Injection Protocol
NSG: Neuroscience Gateway
RN: Input Resistance
\(\tau_m\): Membrane Time Constant
TTX: Tetrodotoxin
VIP-eGFP: VIP/enhanced Green Fluorescent Protein
ACSF: Artificial Cerebrospinal Fluid
OGB-1: Oregon Green® 488 BAPTA-1

Channels:
\(I_{Na,t}\): Transient Sodium Current
\(I_{Na,p}\): Persistent Sodium Current
\(I_{Kdrs}\): Slow Delayed Rectifier Potassium Current
\(I_{Kdrf}\): Fast Delayed Rectifier Potassium Current
\(I_{Ka}\): A-Type Potassium Current
\(I_{CaT}\): T-Type Calcium Current
\(I_{CaL}\): L-Type Calcium Current
\( I_{\text{Noise}} \): Gaussian White Noise Current

**Irregular Firing Equations:**

- **ISI**: Interspike Interval
- **ISI CV**: Coefficient of Variation of the Interspike Interval
- **ISI SD**: Standard Deviation of the Interspike Interval
- **ISI Mean**: Mean Interspike Interval

**Cable Equations Variables:**

- \( C_m \): Specific Membrane Capacitance
- \( c_m \): Membrane Capacitance
- \( R_m \): Specific Membrane Resistance
- \( r_m \): Membrane Resistance
- \( R_a \): Axial Resistance/Specific Intracellular Resistance
- \( r_a \): Cytoplasmic Resistance Between Two Compartments
- \( d \): Diameter of a Cylindrical Compartment
- \( l \): Length of a Cylindrical Compartment
- \( V \): Membrane Potential
- \( t \): Time
- \( x \): Distance Along the Length of the Core Conductor
- \( \tau \): Membrane Time Constant
- \( \lambda \): Membrane Space Constant
- \( I_{\text{ionic}} \): Membrane Currents

**Hodgkin-Huxley Equation Variables:**

- \( E \): Reversal Potential
- \( \hat{G} \): Maximal Conductance
- \( m \) or \( n \): Activation Gate Variables
- \( h \): Inactivation Gate Variable
- \( \alpha \): Forward Rate Constant
- \( \beta \): Backward Rate Constant
- \( V_r \): Resting Membrane Potential
- \( m_\infty \): Steady State Activation
- \( h_\infty \): Steady State Inactivation
- \( \tau_m \): Activation Time Constant
- \( \tau_h \): Inactivation Time Constant

**Euclidean Distance Equation Variables:**

- \( d_{x,y} \): Euclidean Distance between Model and Experimental Measurements
- \( x_i \): Value of Model Measurement \( i \)
- \( y_i \): Value of Experimental Measurement \( i \)
- \( N \): Total Number of Measurements

**Synapse Equation Variables:**

- \( i \): Synaptic Current
- \( G \): Synaptic Conductance
v: Membrane Potential  
e: Reversal Potential  
t: Time  
**Weight:** Synaptic Weight  
**Factor:** NEURON Process Used to Normalize the Peak Synaptic Conductance to 1  
**tau1:** Rise Time  
**tau2:** Decay Time
References


2. Acsády L, Görcs TJ, Freund TF. (1996b). Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. *Neuroscience*. 73(2):317-34.


Appendix A – Channel Equation Details and Plots

Note that all channels are obtained from Lawrence et al, 2006, with the exception of the faster delayed rectifier channel (Saraga et al, 2003) and the persistent sodium channel (Uebachs et al, 2010). All model files for these channels are obtained via ModelDB, an online database of published models that run on a variety of software platforms (Hines et al, 2004).

Transient Sodium:

As described in Lawrence et al, 2006, this channel model was tuned using experimentally determined values from dendritic patch clamp recordings from Oriens/Alveus layer interneurons (Martina et al, 2000) and basket cells gating kinetic measurements from the Dentate Gyrus (Martina and Jonas, 1997). The mathematical structure of the channel equations is based on the original equations for the sodium channel model in Hodgkin and Huxley, 1952.

Transient sodium current:

\[ I_{NaT} = \bar{g}_{NaT} m^3 h (V - E_{Na}) \]

Steady-state activation:

\[ \frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m = \frac{m_{\infty} - m}{\tau} \]

\[ \tau = \frac{1}{\alpha_m + \beta_m}, \quad m_{\infty} = \alpha_m \tau \]

Forward and backward rate functions:

\[ \alpha_m = \frac{-0.1(V + 38)}{\exp\left[\frac{-(V + 38)}{10}\right] - 1}, \quad \beta_m = 4 \exp\left[\frac{-(V + 65)}{18}\right] \]

Steady-state inactivation:

\[ \frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h = \frac{h_{\infty} - h}{\tau} \]

\[ \tau = \frac{1}{\alpha_h + \beta_h}, \quad h_{\infty} = \alpha_h \tau \]
Forward and backward rate functions:

\[
\alpha_h = 0.07 \exp \left[ \frac{-(V+63)}{20} \right], \quad \beta_h = \frac{1}{1+\exp \left[ \frac{-(V+33)}{10} \right]}
\]

Figure A1. Left: Transient sodium steady state activation (blue) and inactivation (green) curves. Right: Transient sodium activation time constants (blue) and inactivation time constants (green).

Persistent sodium:

Although the channel model used here is taken from Uebachs et al, 2010, small adjustments were made to conform to experimentally determined persistent sodium channel kinetics as measured in guinea pig CA1 pyramidal cell layer neurons (French et al, 1990; Skinner et al, 1999; Morin et al, 2010; Sritharan and Skinner, 2012).

Persistent sodium current:

\[ I_{NaP} = \bar{g}_{NaP} m(V - E_{Na}) \]

Steady-state activation:

\[
\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m = \frac{m_\infty - m}{\tau}
\]

\[ \tau = 5 \text{ ms}, \quad m_\infty = \frac{1}{1+\exp \left[ \frac{-(V-51)}{5} \right]} \]
Figure A2. Persistent sodium steady-state activation curve. Note that $m_{\text{tau}} = 5\ \text{ms}$.

A-type potassium:

Like the transient sodium channel, this channel model is obtained from Lawrence et al, 2006 and is based on Hodgkin-Huxley formalism (Hodgkin and Huxley, 1952). As well, this model is tuned according to A-type channel kinetic measurements found in CA1 Oriens-Alveus interneurons (Lien et al, 2002).

A-type potassium current:

$$I_{K_a} = \bar{g}_{K_a}m h (V - E_K)$$

Steady-state activation:

$$\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m = \frac{m_\infty - m}{\tau}$$

$\tau = 0.5\ \text{ms}, \quad m_\infty = \left(\frac{1}{1 + \exp\left[-\left(V+44.4\right)/26.6\right]}\right)^4$

Steady-state inactivation:

$$\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h = \frac{h_\infty - h}{\tau}$$

$\tau = 0.17(V + 105), \quad h_\infty = \frac{1}{1 + \exp\left[V+70.5\right]/6}$
Figure A3. Left: A-type potassium steady state activation (blue) and inactivation (green) curves. Right: A-type potassium activation time constants (blue) and inactivation time constants (green). Note that $m_{\text{tau}} = 0.5$ ms.

Slow delayed rectifier potassium:

Like the A-type potassium channel, this channel model is obtained from Lawrence et al, 2006, is based on Hodgkin-Huxley formalism (Hodgkin and Huxley, 1952) and was tuned according to channel kinetic measurements found in CA1 Oriens-Alveus interneurons (Lien et al, 2002). Note that unlike more traditional formalisms of this channel, the steady state activation, $m$, is not to the power of 4 in the current equation for $I_{K\text{drs}}$. Instead, $m_{\infty}$ is to the power of four. The reason for this is because the experimentally measured slow delayed rectifier activation curve data points were best fit using a Boltzmann function that was raised to the power of four (as mentioned in Lien et al, 2002).

Slow delayed rectifier potassium current:

$$I_{K\text{drs}} = \bar{g}_{K\text{drs}} m_h (V - E_K)$$

Steady-activation activation:

$$\frac{dm}{dz} = \alpha_m (1 - m) - \beta_m m = \frac{m_{\infty} - m}{\tau}$$

$$\tau = \frac{66.7 \exp\left[\frac{V + 25}{14.3}\right]}{1 + \exp\left[\frac{V + 25}{6.7}\right]}$$

$$m_{\infty} = \left(\frac{1}{1 + \exp\left[\frac{-(V + 41.9)}{23.1}\right]}\right)^4$$
Steady-activation inactivation:

\[
\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h = \frac{h_\infty - h}{\tau}
\]

\[
\tau = 1000 \text{ ms}, \quad h_\infty = \frac{0.93}{1 + \exp\left(\frac{V}{15.2}\right)} + 0.07
\]

**Figure A4.** Left: Slow delayed rectifier potassium steady state activation (blue) and inactivation (green) curves. Right: Slow delayed rectifier potassium activation time constants. Note that \(h_{\text{tau}} = 1000 \text{ ms}\).

Fast delayed rectifier potassium:

Like the A-type potassium channel, this channel model is obtained from Lawrence *et al*, 2006, is based on Hodgkin-Huxley formalism (Hodgkin and Huxley, 1952) and was tuned according to channel kinetic measurements in CA1 Oriens-Alveus interneurons (Lien *et al*, 2002). Note that like the slow delayed rectifier model, the steady state activation, \(m\), is not to the power of 4 in the current equation and \(m_\infty\) is instead. Similarly, this is because the fast delayed rectifier activation curve data points were also best fit with a Boltzmann function raised to the power of four (Lien *et al*, 2002).

Fast delayed rectifier potassium current:

\[
I_{K\text{drf}} = \bar{g}_{K\text{drf}} mh(V - E_K)
\]
Steady-activation activation:

\[
\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m = \frac{m_\infty - m}{\tau}
\]

\[
\tau = \frac{27.8 \exp\left[\frac{V + 31}{14.1}\right]}{1 + \exp\left[\frac{V + 31}{10}\right]}, \quad m_\infty = \left(\frac{1}{1 + \exp\left[-\frac{(V + 36.2)}{16.1}\right]}\right)^4
\]

Steady-activation inactivation:

\[
\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h = \frac{h_\infty - h}{\tau}
\]

\[
\tau = 1000 \text{ ms}, \quad h_\infty = \frac{0.92}{1 + \exp\left[-\frac{(V + 60.6)}{7.8}\right]} + 0.08
\]

Figure A5. Left: Fast delayed rectifier potassium steady state activation (blue) and inactivation (green) curves. Right: Fast delayed rectifier potassium activation time constants. Note that \(h_{\tau_1} = 1000 \text{ ms}\).

Faster delayed rectifier potassium:

As mentioned, this channel is a more general delayed rectifier potassium channel used in an older version of an OLM cell model (Saraga et al, 2003). This model uses Hodgkin-Huxley formalism and the steady state activation curves were tuned according to recordings obtained from Oriens/Alveus interneurons (Martina et al, 2000).
Faster delayed rectifier potassium current:

\[ I_{Kdf} = \bar{g}_{Kdf}n^4(V - E_K) \]

Steady-activation activation:

\[
\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n = \frac{n_{\infty} - n}{\tau} \\
\tau = \frac{1}{\alpha_n + \beta_n'} \\
n_{\infty} = \alpha_n \tau
\]

Forward and backward rate functions:

\[
\alpha_n = \frac{-0.018(V - 25)}{\exp\left[\frac{-(V - 25)}{25}\right] - 1}, \quad \beta_n = \frac{0.0036(V - 35)}{\exp\left[\frac{V - 35}{12}\right] - 1}
\]

**Figure A6.** Left: Faster delayed rectifier potassium steady state activation curve. Right: Faster delayed rectifier potassium activation time constants.
Appendix B – Allen Mouse Brain Atlas: Extended Subunit Analysis

Sodium Channel Subunits:

Although there is not much expression of any sodium channel alpha subunits (Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.3 and Na\textsubscript{v}1.6), there is considerable expression of all three types of beta subunits (Na\textsubscript{v}B1.1-Na\textsubscript{v}B 3.1) in the SR layer. These results do not indicate any clear potential balances of transient and persistent sodium channels but suggests that both could be present to some degree.

Figure B1. In situ hybridization data showing genetic expression of SCN1A (i.e. the gene that encodes Na\textsubscript{v}1.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=74735074&imageId=74654652&initImage=ish&coordSystem=pixel&x=5753.14553869517&y=3252.7068292677623&z=3
Figure B2. In situ hybridization data showing genetic expression of SCN2A (i.e. the gene that encodes Na$_v$1.2 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=100145300&imageld=102240148&initimage=ish&cordsystem=pixel&x=6763.85453325701&y=3301.768455631134&z=5

Figure B3. In situ hybridization data showing genetic expression of SCN3A (i.e. the gene that encodes Na$_v$1.3 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:
Figure B4. In situ hybridization data showing genetic expression of SCN8A (i.e. the gene that encodes Na\textsubscript{v}1.6 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramide, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=69863261&imageId=69884064&initImage=ish&coordSystem=pixel\&x=7315.375567650341\&y=3442.7785724578453\&z=3

Figure B5. In situ hybridization data showing genetic expression of SCN1B (i.e. the gene that encodes Na\textsubscript{v}B1.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-
Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=77454673&imageId=77452482&initImage=ish&coordSystem=pixel&x=5895.3762225237015&y=2807.605896838878&z=3

Figure B6. In situ hybridization data showing genetic expression of SCN2B (i.e. the gene that encodes Na,B2.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=73573157&imageId=73515617&initImage=ish&coordSystem=pixel&x=6038.922405574622&y=3463.6744689952216&z=3
Figure B7. In situ hybridization data showing genetic expression of SCN3B (i.e. the gene that encodes Na\textsubscript{v}B3.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=68797529&imageId=68392543&initImage=ish&coordSystem=pixel&x=6081.20125823218&y=3281.1047067898526&z=3

A-Type Potassium Channel Subunits:

As mentioned, potential A-type potassium subunits include K\textsubscript{v}1.4, K\textsubscript{v}3.3, K\textsubscript{v}3.4, K\textsubscript{v}4.1, K\textsubscript{v}4.2 and K\textsubscript{v}4.3. Previous studies have shown that K\textsubscript{v}4.3 subunits are highly expressed in the CA1 SO layer (Lien et al., 2002). In fact, it is the channel kinetic measurements taken from this study that constrain the A-type potassium channel model used in our IS3 cell model as well as in previous OLM cell models (Saraga et al., 2003; Lawrence et al., 2006).

Using Allen Mouse Brain Atlas, we see that it is mostly only K\textsubscript{v}4.3 subunits that are expressed in the SR and SLM layers, which is in line with the kinetics of the A-type potassium channel model that is used in the IS3 cell model.

Figure B8. In situ hybridization data showing genetic expression of KCNA4 (i.e. the gene that encodes K\textsubscript{v}1.4 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:
Figure B9. In situ hybridization data showing genetic expression of KCNC3 (i.e. the gene that encodes Kv3.3 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:
Figure B10. In situ hybridization data showing genetic expression of KCNC4 (i.e. the gene that encodes K,3.4 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=1756&imageId=101366509&initImage=ish&coordSystem=pixel&x=5085.30717604214&y=2294.1696537020525&z=3

Figure B11. In situ hybridization data showing genetic expression of KCND1 (i.e. the gene that encodes K,4.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:
Figure B12. In situ hybridization data showing genetic expression of KCND2 (i.e. the gene that encodes $K_{v}4.2$ subunits) in the CA1 area of the hippocampus ($slm =$ Stratum Lacunosum-Moleculare, $sr =$ Stratum Radiatum, $sp =$ Stratum Pyramidal, $so =$ Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=69258017&imageId=69147778&initImage=ish&coordSystem=pixel&x=5784.786623429449&y=2850.822416190175&z=3

Figure B13. In situ hybridization data showing genetic expression of KCND3 (i.e. the gene that encodes $K_{v}4.3$ subunits) in the CA1 area of the hippocampus ($slm =$ Stratum Lacunosum-Moleculare, $sr =$ Stratum Radiatum, $sp =$ Stratum Pyramidal, $so =$ Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=69258011&imageId=69147731&initImage=ish&coordSystem=pixel&x=6083.305156120161&y=2963.28799632143&z=3
Slow Delayed Rectifier Potassium Channel Subunits:

As mentioned previously, slow delayed rectifier potassium channels seem to mainly be composed of \( K_{v2.1} \) or \( K_{v2.2} \) subunits in combination with \( K_{v5-9} \) subunits (e.g. \( K_{v5.1}, K_{v6.4}, K_{v9.2}, \) or \( K_{v9.3} \)). Here we show that none of these subunits have much expression in the SR and SLM CA1 layers, if any. Primarily, we see that \( K_{v2.1} \) and \( K_{v5.1} \) have by far the largest amount of expression in the SP and SO layers, and so these subunits may likely form heteromeric complexes in these regions. This high expression stops sharply at the SP/SR border with both subunits not being expressed at all in the SR layer and only exhibiting mild, sparse expression in the SLM layer. All the other subunits only display very mild expression in CA1. Similarly to \( K_{v2.1} \) and \( K_{v5.1} \), \( K_{v9.3} \) also shows mild and sparse expression in the SLM layer. None of the slow delayed rectifier subunits show any expression in the SR layer, suggesting that the slow delayed rectifier channel may not be present in IS3 cells.

Figure B14. In situ hybridization data showing genetic expression of \( KCNB1 \) (i.e. the gene that encodes \( K_{v2.1} \) subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:
Figure B15. In situ hybridization data showing genetic expression of KCNB2 (i.e. the gene that encodes K₂.2.2 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=74581424&imageId=74478097&initImage=ish&coordSystem=pixel&x=8197.816475114992&y=3758.436971298406&z=3

Figure B16. In situ hybridization data showing genetic expression of KCNF1 (i.e. the gene that encodes K₅.1 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-
Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=71358578&imageId=71153752&initImage=ish&coordSystem=pixel&x=7971.6864765208165&y=3762.147701491181&z=3

Figure B17. In situ hybridization data showing genetic expression of KCNG4 (i.e. the gene that encodes Kv6.4 subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidale, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=72081560&imageId=71981464&initImage=ish&coordSystem=pixel&x=11761.393702880809&y=5249.397470741307&z=3
Figure B18. In situ hybridization data showing genetic expression of KCNS2 (i.e. the gene that encodes K\(_{\text{v}9.2}\) subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:


Figure B19. In situ hybridization data showing genetic expression of KCNS3 (i.e. the gene that encodes K\(_{\text{v}9.3}\) subunits) in the CA1 area of the hippocampus (slm = Stratum Lacunosum-Moleculare, sr = Stratum Radiatum, sp = Stratum Pyramidal, so = Stratum Oriens). Image credit: Allen Institute for Brain Science. URL:

http://mouse.brain-map.org/experiment/siv?id=77371817&imageId=77376002&initImage=ish&coordSystem=pixel&x=8199.351891086993&y=3748.3674965275663&z=3
Appendix C – Extended Electrophysiological Measures in PANDORA

Table C1. Descriptions of PANDORA measurements used for each current injection protocol (CIP). Note that measurement names marked by an asterisk were added to PANDORA manually. The right column shows these measurements for the “canonical” trace shown in figure 2, the top models from cases 7 and 8 as well as the case 8* and 9* models.

<table>
<thead>
<tr>
<th>-100 pA CIP Measurements</th>
<th>Measurement Description</th>
<th>Cell # 14227002 (Canonical)</th>
<th>Case 7 Top Model</th>
<th>Case 8 Top Model</th>
<th>Case 8*</th>
<th>Case 9*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PulsePotMin (mV)</td>
<td>The minimum membrane potential obtained during hyperpolarization</td>
<td>-112.6709</td>
<td>-112.4475</td>
<td>-104.5453</td>
<td>-112.2973</td>
<td>-112.4038</td>
</tr>
<tr>
<td>PulsePotMinTime (ms)</td>
<td>The time at which the membrane potential reaches its minimum during hyperpolarization</td>
<td>58.9000</td>
<td>377.6000</td>
<td>273.9000</td>
<td>357.6000</td>
<td>357.6000</td>
</tr>
<tr>
<td>PulsePotSag (mV)</td>
<td>The amount of sag (mV) exhibited by the trace as a result of hyperpolarization</td>
<td>6.7865</td>
<td>0.7467</td>
<td>0.7885</td>
<td>0.5638</td>
<td>0.5632</td>
</tr>
<tr>
<td>PulsePotTau (ms)</td>
<td>The time constant for fitting an exponential curve to the decay of the hyperpolarization-induced sag.</td>
<td>14.3000</td>
<td>27.4000</td>
<td>16.9000</td>
<td>27.4000</td>
<td>27.2000</td>
</tr>
<tr>
<td>PulseIniSpontPotAvgDiff* (mV)</td>
<td>The difference between the average membrane potential during the current injection and the average membrane potential preceding the current injection</td>
<td>-34.2649</td>
<td>-29.4468</td>
<td>-39.6985</td>
<td>-40.4222</td>
<td>-29.4468</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>20 pA CIP Measurements</th>
<th>Measurement Description</th>
<th>Cell # 14723001 (Canonical)</th>
<th>Case 7 Top Model</th>
<th>Case 8 Top Model</th>
<th>Case 8*</th>
<th>Case 9*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PulseIniSpontPotAvgDiff* (mV)</td>
<td>The difference between the</td>
<td>12.4910</td>
<td>8.1472</td>
<td>3.8658</td>
<td>8.1154</td>
<td>9.9154</td>
</tr>
</tbody>
</table>
average membrane potential during the current injection and the average membrane potential preceding the current injection

<table>
<thead>
<tr>
<th>50 pA CIP Measurements</th>
<th>Measurement Description</th>
<th>Cell # 14227002 (Canonical)</th>
<th>Case 7 Top Model</th>
<th>Case 8 Top Model</th>
<th>Case 8*</th>
<th>Case 9*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PulseFirstSpikeTime (ms)</td>
<td>The amount of time between the start of the current injection and the first spike</td>
<td>33.1000</td>
<td>75.1000</td>
<td>213.5000</td>
<td>41</td>
<td>25.4000</td>
</tr>
<tr>
<td>PulseSFA</td>
<td>The spike frequency accommodation of the interspike interval during the current injection</td>
<td>1.3305</td>
<td>1.5949</td>
<td>1.0444</td>
<td>0.6633</td>
<td>0.7590</td>
</tr>
<tr>
<td>PulseSpikeRate (Hz)</td>
<td>The firing frequency during the current injection</td>
<td>35.0044</td>
<td>60.0075</td>
<td>11.2514</td>
<td>65.0081</td>
<td>76.2595</td>
</tr>
<tr>
<td>PulseSpikeRateISI (ms)</td>
<td>The mean interspike interval between spikes during the current injection</td>
<td>36.6898</td>
<td>65.0069</td>
<td>14.4823</td>
<td>68.5116</td>
<td>78.0539</td>
</tr>
<tr>
<td>PulseSpikeAmplitudeMean (mV)</td>
<td>The mean amplitude of the spikes during the current injection. Amplitude is calculated as the difference from the spike initiation point (i.e. the point of maximum curvature in the V-dV/dt phase plane) to the peak of the spike</td>
<td>61.7296</td>
<td>61.8073</td>
<td>71.2885</td>
<td>58.7644</td>
<td>54.4863</td>
</tr>
<tr>
<td>PulseSpikeHalfWidthMean (mV)</td>
<td>The average width of the spikes at the point where the membrane potential is equal to half of the</td>
<td>1.0301</td>
<td>1.2825</td>
<td>1.4076</td>
<td>1.3252</td>
<td>1.3922</td>
</tr>
<tr>
<td>Measurement</td>
<td>Description</td>
<td>Cell # 14227002 (Primary)</td>
<td>Case 7 Top Model</td>
<td>Case 8 Top Model</td>
<td>Case 8*</td>
<td>Case 9*</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>500 pA CIP Measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PulseIni100msSpikeRate (Hz)</td>
<td>The firing rate in the initial 100ms of the current injection</td>
<td>60.0000</td>
<td>30</td>
<td>70</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>PulseIni100msRestIniSpatPotAvgDiff* (mV)</td>
<td>The difference between the average membrane potential after the first 100ms of the current injection and the average membrane potential preceding the</td>
<td>62.6461</td>
<td>34.5502</td>
<td>45.3177</td>
<td>35.9546</td>
<td>35.1328</td>
</tr>
</tbody>
</table>
Experimental -100pA CIP Measurements:

-100 pA Measure Histogram for PulsePotMin

-100 pA Measure Histogram for PulsePotMinTime

-100 pA Measure Histogram for PulsePotSag

-100 pA Measure Histogram for PulsePotTau

-100 pA Measure Histogram for PulsePotPotAvgDiff
Experimental 20pA CIP Measurements:

![Histogram for PulseFirstSpikeTime](image)

![Histogram for SpikeRate](image)

Experimental 50pA CIP Measurements:

![Histogram for PulseFirstSpikeTime](image)

![Histogram for SpikeRate](image)
**Experimental 500pA CIP Measurements:**

Figure C1. Experimental PANDORA measurement histograms. The dashed red line represents the measurement values of the canonical traces (as listed in table C1) shown in figure 2. The green, magenta, black and yellow dashed lines represent the measurement values of the case 7 top model, the case 8 top model, the case 8* model and the case 9* model, respectively, as they are listed in table C1. Note that because of limited experimental data, experimental histograms are generated from data where the only criterion is that they exhibit regular spiking regimes (i.e. current injection protocols range from 10pA to 140pA for the spiking regime).
Appendix D – PSIBAuRg: Cases 10-12

Channel distributions are summarized in table D1 and results are summarized in figure D1 and table D2. In general, these cases generate appropriate depolarization heights in membrane potential during depolarizing current injections, but at the cost of other measurements such as the first spike time, spike amplitude adaptation and spike amplitude. In other words, the inclusion of calcium channels does not significantly improve the models from the 7-9 cases. In any case, the conductance ranges are similar to what is seen in cases 7-9 (table A2).

Table D1. Summary of channel type combinations and spatial distribution profiles across the morphology of the model for cases 10-12 (i.e. with $I_{CaL}$ and $I_{CaT}$). Note that in all cases, each channel has a uniform distribution, whether it be restricted to the soma or distributed across the soma and dendrites. Also note that “static” simply implies that the conductance value is not adjusted during the parameter search (i.e. the channels are held at one conductance value).

<table>
<thead>
<tr>
<th>Case #</th>
<th>Soma Channel Types</th>
<th>Dendrite Channel Types</th>
<th>Axon Channel Types</th>
</tr>
</thead>
</table>
| Case 10 | Persistent Sodium  
Transient Sodium  
A-Type Potassium  
Faster Delayed Rectifier Potassium  
L-Type Calcium (Static)  
T-Type Calcium (Static) | None | None |
| Case 11 | Persistent Sodium  
Transient Sodium  
A-Type Potassium  
Faster Delayed Rectifier Potassium  
L-Type Calcium (Static)  
T-Type Calcium (Static) | Transient Sodium  
A-Type Potassium  
Faster Delayed Rectifier Potassium  
L-Type Calcium (Static)  
T-Type Calcium (Static) | None |
| Case 12 | Persistent Sodium  
Transient Sodium  
A-Type Potassium  
Faster Delayed Rectifier Potassium  
L-Type Calcium (Static)  
T-Type Calcium (Static) | Transient Sodium  
Faster Delayed Rectifier Potassium  
L-Type Calcium (Static)  
T-Type Calcium (Static) | None |
Table D2. Summary of the appropriate conductance ranges found using the PSIBAuRg approach for cases 10-12.

<table>
<thead>
<tr>
<th>Case #</th>
<th>(G_{Na}^t) (S/cm(^2))</th>
<th>(G_{Na,p}^t) (S/cm(^2))</th>
<th>(G_{Ka}) (S/cm(^2))</th>
<th>(G_{Kdrf}) (S/cm(^2))</th>
<th>(G_{Ca,T}) (S/cm(^2))</th>
<th>(G_{Ca,L}) (S/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.14-0.18</td>
<td>0.00005-0.00015</td>
<td>0.3-0.5</td>
<td>0.7-0.8</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>11</td>
<td>0.04-0.05</td>
<td>0.0002-0.0003</td>
<td>0.05-0.07</td>
<td>0.1-0.12</td>
<td>0.0002</td>
<td>0.0001</td>
</tr>
<tr>
<td>12</td>
<td>0.03-0.05</td>
<td>0.0003-0.0005</td>
<td>0.5-0.7</td>
<td>0.22-0.26</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Case 10

Case 11
Case 12

Figure D1. LEFT: Top models in cases 10-12 (from top to bottom). Current injection protocol is the same as what is seen in figure 2. RIGHT: Parameter spaces for cases 10-12 (from top to bottom), as visualized using clutter based dimensional reordering. Each pixel represents a single model and that model’s distance metric. Conductance axes are organized such that overall low conductance models are in the bottom left quadrants and overall high conductance models are in the top right quadrants. Black pixels represent models that are rejected during pre-processing and are assigned a distance value of 100 as a result.