Investigations of microcircuitry in the rat barrel cortex using an experimentally constrained layer V pyramidal neuron model

Inaugural – Dissertation

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Jonas Dyhrfjeld-Johnsen

aus Kopenhagen

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1 Introduction

The mammalian neocortex consists of developmentally determined repeating units known as *minicolumns*, which in primates contain ~80-100 neurons synaptically linked across the six cortical layers (Buxhoeveden et al., 2000; Mountcastle, 1997). These minicolumns are further grouped by local connections into *columns* also known as *modules*, since their constituent neurons have common response properties and are considered the functional units of the neocortex. The columnar structure results from both intracortical circuitry and termination patterns of afferent projections.

This characteristic structural organization is most easily investigated in, but not limited to, primary sensory cortices, where it has been possible to map the response to presented peripheral stimuli (visual, somatosensory, auditory) in anaesthetized animals with microelectrodes (Mountcastle, 1997). When penetrating the cortex perpendicular to the pial surface and moving the microelectrode down through the cortical layers, neurons within a column responding to common adequate stimuli are encountered. When moving an electrode parallel to the pial surface, blocks of neurons with common response properties (corresponding to adjacent columns) follow each other with sharp boundaries separating them. In primary sensory cortices, the stimulus representation is topographically organized in maps with adjacent columns receiving input originating from neighbouring peripheral receptors. Such a mapping conserves the relationship between peripheral stimuli in a manner facilitating both neighbourhood-based feature extraction and integration of input in a behaviourally relevant context (Diamond et al., 1999; Kaas, 1997).

Whereas the receptive fields of primary sensory cortices are relatively well mapped, the detailed interactions between local neuronal populations in the microcircuitry defining the functional properties of the columns are still a major area of investigation (Thomson & Deuchars, 1997; Thomson & Bannister, 2003; Staiger et al., 2000). In other words, there is a good understanding of *where* and *what* but not of *how*. A better understanding of the properties of such local networks and how they integrate, process and code information is essential to understanding the functional properties of cortical areas. A very important step in these investigations lies in establishing the functional connectivity of the different neuron types involved in the local microcircuits.

In rodents, the posteromedial barrel field of the somatosensory cortex receiving sensory input from the large facial whiskers or *vibrissae* of the animal has particularly large and easily identifiable columns with well affererent connections, making it an ideal system for investigating the structure-function relationships in cerebral cortex (Diamond et al., 1999).

1.1 The rat barrel system

The term *barrel cortex* was coined by Woolsey and Van der Loos (1970) reflecting the typical appearance of cytochrome oxidase staining in layer IV of rodent primary somatosensory cortex receiving sensory input from the facial whiskers of the animal. The neurons in layer IV (the granular layer) are grouped in a barrel-like appearance, forming a higher cell density ring surrounding a sparser populated barrel center. These barrels are particularly large in the posteromedial barrel field (PMBF). The repeated barrel structures, separated by septa, are not only clearly visible in different stainings of cortical slices, but also distinguishable in the unstained living slice (Agmon & Connors, 1991; Kötter et al., 1998; Schubert et al., 2001).



Fig. 1.1: A. Schematic illustration of the location of barrel cortex in the brain. Directions signify anterior (A), postererior (P) as well as medial (M) and lateral (L) only for the right hemisphere. B. A cytochrome oxidase stained tangential (parallel to brain surface) slice clearly shows the barrel-like structure. Scalebar is 1 mm (Modified from Jablonska et al. (1999) and Brett-Green et al. (2001)).

Each barrel has been shown to receive its primary input from one contralateral whisker, with this one-to-one correspondence also reflected in the somatotopical organization of the barrels (Jones & Diamond, 1995; Figure 1.2). The individual large hairs of the rat whiskers are arranged in matrix-like manner with 5 rows and 5-9 columns on the upper lip (Figure

1.2). The rows are assigned letters (A, B, C, D, and E) from dorsal to ventral, whereas the columns are numbered from caudal to rostral beginning with 1.



Fig. 1.2: The whisker matrix on the rat snout is somatotopically represented by the barrels in the primary somatosensory cortex (Modified from Sherburn et al., 1999).

These extremely mobile and sensitive whiskers are employed by the rat for spatial orientation (Brecht et al., 1997) as well as active exploration of objects and surfaces in the environment and can be used to distinguish surface textures in great detail (Carvell & Simons, 1990). The importance of the vibrissal system for rats as nocturnal animals with poor vision is reflected by the proportions of the barrel field representation in the somatosensory cortex relative to the rest of their body.

1.2 The whisker to barrel pathway

Whisker deflections innervate receptor neurons in the whisker follicles projecting primarily to the *trigeminal nucleus principalis* (PrV) in the *trigeminal nuclear brainstem complex* (TNBC) by way of the infraorbital branch of the trigeminal (V) nerve (Waite & Tracey, 1995). Similar to the described correspondence of whiskers to barrels in the cortex, PrV contain cell patches named *barellettes* responding primarily to one principal whisker (Henderson & Jacquin, 1995; Tracey & Waite, 1995).

Areas in the TNBC receiving whisker input project to parts of the contralateral thalamus. Neurons in the *medial* part of thalamic *ventral posterior nucleus* (VPm) receiving input from PrV are arranged in cell assemblies named *barreloids*, again associated with one primary whisker thus preserving the topographic mapping of the peripheral input (Haidarliu & Ahissar, 2001; Diamond, 1995). This *lemniscal* pathway via the VPm primarily projects to the barrel centers in layer IV of the barrel cortex, but also to some degree to lower layer III as well as the border between layers Vb and VI (Arnold et al., 2001; Lu & Lin, 1993; Waite & Tracey, 1995). The *medial* part of the *thalamic posterior nucleus* (POm) receives a more diffusely terminating input from the brainstem and projects in what is known as the *para-lemniscal* pathway to the inter-barrel septa in layer IV, as well as layers I and Va (Lu & Lin, 1993; Waite & Tracey, 1995). The paralemniscal input from POm to the barrel cortex generally shows longer latencies than the lemniscal input from VPm (Ahissar et al., 2000; Diamond et al., 1992).

Whereas the PrV neurons in the brainstem has been shown *in vivo* to relay information without any apparent transformation of their afferent input (Ahissar et. al, 2000), information processing in the whisker to barrel pathway already takes place at the level of VPm and POm in the thalamus, with the lemniscal pathway via VPm conveying temporal aspects of the peripheral stimulus, while the paralemniscal pathway via POm is involved in processing spatial features (Ahissar et al., 2000; Sosnik et al., 2001). Activity in both thalamic nuclei is additionally strongly modulated by feedback projections from the infragranular barrel cortex (Ahissar et al., 2000; Waite & Tracey, 1995).

1.3 Structure of the barrel cortex

As previously described, the information processing takes place in columnar modules of neurons interconnected across the six cortical layers. In the barrel cortex, neurons above and below the distinctive layer IV (granular layer) barrels are activated by stimulation of the barrel-associated whisker (Simons, 1995), with intermediate latencies in the supragranular layers and longer latencies in the infragranular regions (Zhu & Connors, 1999). Both supra- and infragranular neurons have larger receptive fields than the layer IV neurons comprising the barrels, showing stronger responses to stimulation of adjacent whiskers. Along with neurons in the supra- and infragranular layers, the layer IV neurons constitute what is often termed a *barrel-related column* of neurons associated with a common principal whisker.



Fig. 1.3: The repeated barrel structures in layer IV are clearly visible in a cytochrome oxidase stained coronal cortex (perpendicular to the brain surface) slice of the barrel. Scale bar 500 µm (From Lübke et al., 2000).

This structure and sequence of activation has led to the idea of a *feed forward* activation of the barrel cortex, with a sequence of activation following the path from layer IV over layers II/III to layer V and VI (Staiger et al., 2000). However, studies of connectivity of single neurons in the different layers of the barrel cortex give a more complicated picture of the columnar function, as will be discussed below.

1.4 Neuronal populations in the barrel cortex

Two main groups of neurons comprise the microcircuitry of the cortex: Inhibitory interneurons primarily using γ -aminobutyric acid (GABA) as the main neurotransmitter are characterized by their smooth or sparsely spiny dendrites, whereas the principal excitatory neurons transmit signals using the neurotransmitter L-glutamate and have dendrites covered by spines (Keller, 1995; Mountcastle, 1997; Thomson & Deuchars, 1997; Thomson & Bannister, 2003). Both groups contain neurons with different morphological and physiological characteristics, and are differentially distributed in the cortical layers.

1.4.1 Inhibitory interneurons

The inhibitory neurons comprise a large group of morphologically distinct cell types distributed throughout the cortical layers, displaying a large variety of action potential firing patterns (Gupta et al., 2000). The *fast spiking* (FS) capable of firing trains of high

frequency action potentials is the most common, but some also show burst firing or more complex patterns (Connors & Gutnick, 1990; Gupta et al., 2000). The strongest inhibitory connections are made on targets in the local cortical column targeting both interneurons and the principal excitatory neurons with somewhat weaker connections also made across columnar borders implicating their involvement in both modulation of local activity as well as possibly influencing the activity patterns in the cortex on a larger scale (Nicoll et al., 1996; Salin & Prince, 1996). Inhibitory interneurons receiving direct thalamic inputs have been shown to play a large role in determining the period where activation of the excitatory circuitry in a column can occur, in effect invoking a type of filter sorting out weak or irrelevant information (Porter et al., 2001; Swadlow, 2003). In spite of their obvious importance in the local circuitry, the connectivity of the very different subclasses of interneurons is not well established.

1.4.2 Excitatory neuronal populations

The spiny excitatory neurons can be more easily grouped according to their intrinsic properties than the inhibitory interneurons, firing either short bursts or regular trains of action potentials after supra-threshold depolarization (Connors & Gutnick, 1990). The excitatory neurons fall into two groups according to their morphological characteristics: Pyramidal or spiny stellate neurons.

The spiny stellate neurons are only found in layer IV of the barrel cortex, where they make up ~80% of the excitatory neuronal population with the remaining ~20% consisting of star pyramidal neurons (Lübke et al., 2000). The spiny stellates have spherical or ovoid cell bodies and relatively compact multipolar dendritic tree almost exclusively confined to the home barrel. Named after their pyramid shaped cell bodies, the star pyramidal neurons show a prominent non-tufted apical dendrite ascending to layer II/III in addition to their basal dendrites that stay within the home barrel. Both neuron types can display either regular spiking or intrinsically bursting behaviour (Schubert et al., 2003).

Layers II and III (supragranular layers) contain pyramidal neurons with basal dendrites staying within the supragranular layers and an apical dendrite with numerous minor branches extending towards to the pial surface (Gottlieb & Keller, 1997). The layer II/III pyramidal neurons display mostly RS action potential firing patterns and have corticocortical projections to areas in the same hemisphere such as secondary

somatosensory and primary motor cortex (Keller, 1995; Kelly et al., 2001; Kim & Ebner, 1999).

In the infragranular layers, layer Vb contains neurons of the large pyramidal type with long apical dendrites reaching into the superficial cortical layers (II and I) where they form a terminal tuft (Gottlieb & Keller, 1997; Thomson & Bannister, 1998) with IB neurons having more extensively branched dendrites both in the apical and basal domain (Schubert et al., 2001). In addition to the larger pyramidal cells in layer Vb, the thinner upper subdivision of this layer (Va) contains smaller pyramidal neurons with a shorter apical dendrite. Beside associative corticocortical connections, the larger layer Vb pyramidal neurons also provide feedback connections to subcortical structures like the striatum, superior colliculus and brainstem and to a lesser extent the thalamus (Deschênes et al., 1994; Staiger et al., 1999; Waite & Tracey, 1995). The main source of feedback to the thalamus originates from layer VI pyramidal neurons, which also supply some corticocortical connections (Zhang & Deschênes, 1997; Waite & Tracey, 1995).

1.4.3 Intracolumnar connectivity

Processing of the sensory input from the primary associated whisker in a barrel-associated column happens through a combination of intra- and translaminar connections between the neurons within the column. Although thalamic afferents not only terminate in the barrels, but also on the border between layer V and VI, in layer I and layer Va (see section 1.2), the activation of the columnar circuitry is still predominantly initiated by the VPm projections to layer IV (Moore & Nelson, 1998; Petersen & Sakmann, 2001). This activation is then fed through the described simplified pathway from layer IV over supragranular to infragranular layers where most of the projections to other cortical and subcortical structures originate (Waite & Tracey, 1995). The axonal projections serving as the underlying framework for this feed forward transmission of activity have been established in tracing studies as well as the functional connectivity of individually connected neurons (Feldmeyer et al., 2002; Gottlieb & Keller, 1997; Lübke et al., 2000; Thomson & Bannister, 1998). Neurons on each of the levels of this feed forward pathway are also strongly interconnected within their separate layers (Feldmeyer et al., 1999; Markram et al., 1997; Petersen & Sakmann, 2000; Schubert et al., 2001; Schubert et al., 2003, Thomson & Bannister, 1998). Conversely, the characteristics of feedback connections has so far only been described in much less detail, primarily at the level of axonal tracing

studies and mapping of presynaptic neuron locations (Gottlieb & Keller, 1997; Schubert et al., 2003; Staiger et al., 2000; Zhang & Deschênes, 1997).



Fig. 1.4: The complex local connectivity in the barrel cortex as reported from a variety of experimental investigations spanning from dual recording of synaptically coupled neurons over studies mapping presynaptic neurons to axonal tracing studies (arrowheads do not indicate location of individual synapses). The primarily local inhibitory connections of the highly inhomogeneous population smooth interneurons are not well defined.

1.4.4 Transcolumnar connectivity

Connections between neurons in different columns of sensory cortices serve to integrate individual inputs into behaviourally relevant contexts. In the barrel cortex, the input from a single whisker on the snout covers only a small discrete area of the surface or object being explored, and must be put in relation to the input from adjacent whiskers. Identified connections that cross the columnar borders are particularly prominent in the supra- and infragranular layers (Feldmeyer et al., 2002; Feldmeyer & Sakmann, 2000), and axonal tracing studies have established that these connections can extend horizontally up to seven columns with the densest arborizations in the neighbouring columns (Gottlieb & Keller, 1997). Additionally, recent mapping studies have identified connections between layer IV

neurons in neighbouring barrels specifically targeting star pyramidal neurons (Schubert et al., 2003).

These connections serve as the basis for neuronal responses to other whiskers than the one providing the primary input to their home barrel, thus to a large extent determining the size of the neuronal receptive fields (Ghazanfar & Nicolelis, 2001; Mirabella et al., 2001; Zhu & Connors, 1999). Activations from secondary and tertiary whiskers generally have smaller amplitudes and larger latencies than input from the primary whisker. Transcolumnar connections also play an important role in plasticity of the cortex, which has been shown in experiments investigating the changes in columnar response properties to neighbouring whisker input after clipping of the primary whisker in adult rats (Diamond et al., 1999; Fox, 2002).

1.4 The role of layer V intrinsically bursting pyramidal neurons

The large layer V intrinsically bursting pyramidal neurons of the barrel cortex form reciprocical excitatory synaptic connections with spiny neurons in all other cortical layers (Figure 1.4) and are additionally sources and targets of long ranging horizontal connections spanning up to seven cortical columns (Gottlieb & Keller, 1997), making them prime candidates for integrating a large number of different synaptic inputs. With their ability to repeatedly fire short, high frequency bursts of action potentials, they have been implicated in the generation of synchronized cortical oscillatory activity believed to be the basis for binding several features into a unified cortical representation (Jones & Barth, 1997; Singer, 1993). Since the explorative process employing the whiskers is active rather than passive (Carvell & Simons, 1990), the projections from layer V IB neurons to subcortical structures like the brainstem and superior colliculus are necessary to modulate ongoing whisking behavior (Cauller, 1995).

Behavorial studies investigating the effects of cortical lesions have shown that the supragranular layers are essential for the ability to acquire, but not perform, tasks involving sensory discrimination (Diamond et al., 1999). This learning mechanism involves feedback projections from other cortical areas such as secondary somatosensory and primary motor cortex onto the distal apical dendrites of layer V IB pyramidal neurons as well as pyramidal neurons in layer II/III (Cauller, 1995; Cauller & Connors, 1994).

1.5 Scope of this thesis

Intrinsic electrophysiological properties, such as specific membrane resistance and distributions of different voltage-gated conductances, have been shown to vary dramatically between different subdomains of the dendrites of layer V pyramidal neurons (Larkum & Zhu, 2002; Larkum et al., 2001; Stuart & Spruston, 1998; Williams & Stuart, 2000). These inhomogeneous distributions, along with the position of inhibitory synapses, have been shown to play a large role in determining responses to excitatory stimulations at different positions in the dendritic tree (Berger & Lüscher, 2003; Larkum et al., 1999). These findings indicate that the exact positions of individual synapses could play a large role in the functional properties and information processing of layer V IB pyramidal neurons. So far, only the connectivity of neighboring layer V IB neurons has been described experimentally at this level of detail using a combination of paired recordings from connected neurons and ultrastructural studies of individual synaptic positions (Markram et al., 1997).

To investigate the distributions of synaptic contacts from different presynaptic neuron populations, a detailed realistic model of a layer V IB pyramidal neuron was constructed, based on a reconstructed morphology from experiments investigating the origins of local synaptic inputs to single identified neurons (Schubert et al., 2001). Such modelling approaches have been proven highly fruitful in investigating the interactions between intrinsic neuronal properties and synaptic inputs in other complex neurons like the cerebellar Purkinje cells (De Schutter & Bower, 1994b; Jaeger et al., 1997). As this type of modelling in highly dependent on detailed experimental data for both implementation and testing of models, the database CoCoDat (*Collection of Cortical Dat*a) was implemented in Microsoft Access to facilitate organization and structured retrieval of data reported in the literature.

The results of the modelling and analysis of responses to stimulation of different presynaptic neuron populations described in recent experiments (Schubert et al., 2001) indicates a polarization of synaptic locations on the layer V IB pyramidal neurons made by connections from the local microcircuitry. Seen in the context of previous reports of restriction of input from cortical areas to the distal dendrites of the layer V IB neurons, this indicates a correlation between segregation of synapse locations and the intrinsic properties of the dendritic subdomains they are made on.

2 CoCoDat: A database of quantitative single neuron and microcircuitry data

A major component of constructing and implementing realistic single neuron models is collating, organizing and selecting appropriate experimental data from the published literature. Many reports provide only a fraction of useful data on a particular entity like e.g. an ionic current, and a complete characterization must be assembled from several sources. Furthermore, data on a particular cell to be modelled might not be available in the literature, and the adaptation of data from a "closest possible match" is necessary for construction of a functioning model. In all cases, a large effort goes into organizing, comparing and analyzing the reported experimental data, as well as the experimental methods used to obtain them, to select the optimal empirical values for the model in question.

In recent years, several databases have been constructed and made publicly available, that to some extent facilitate the work of the modeller (for overview see: Chicurel 2000, Kötter 2001, Kötter 2002). However the problems of inhomogeneous non-standardized datasets pose the problem of how to efficiently represent data sets in a database. Existing neuronal databases have dealt with this problem by focusing on single areas of information like morphology (Ascoli et al., 2001; Cannon et al., 1998; Gonzalez et al., 2001), single species (Stein et al. 2001) or a qualitative representation of single neuron features (Mirsky et al., 1998). Thus, the modeller would have to either use several different databases to obtain all necessary information, or take the database as a rough guide before turning to the individual reports for more detailed or quantitative data.

The relational database CoCoDat (*Co*llation of *Co*rtical *Dat*a) (D.-Johnsen et al., 2003) is designed as an advanced tool for organizing published single neuron data for modelling purposes. Non-interpreted quantitative data characterizing features of single neurons and microcircuits, as well as quoted descriptive information, is stored and linked to detailed bibliographical and methodological information adhering strictly to the terminology originally used in the entered publications. All data are linked to a precisely defined *RecordingSite* containing information on the *BrainRegion, Layer, NeuronType* and *NeuronCompartment* the information is acquired from.

CoCoDat is equipped with powerful search functions allowing the user to perform combinatorial queries using the specifications of *RecordingSite*, *DataCategory* and *Species*.

2.1 Design objectives

When designing the CoCoDat database, the main goal was to implement a flexible tool for systematically organizing and extracting datasets for biophysically realistic models. Specifically, the design was based on the following four main objectives: (i) Applicability of the methodology to the broad variety of published data, (ii) maintenance of transparency, (iii) flexibility for further development, and (iv) implementation of powerful search and representation tools for guiding modelling efforts. To achieve these objectives, the following was done:

- The tables constituting the database were derived from a comprehensive survey of representative electrophysiological and anatomical literature from the last ~15 years, ensuring that tables and their fields in CoCoDat reflect the information and values commonly reported in publications.
- Transparency is maintained by the strict 1:1 representation of published data, as well as detailed referencing of the source of data (including exact page and figure numbers). By avoiding any analysis or interpretation of entered data, the entries of CoCoDat are faithful representations of the original descriptions in the literature. Furthermore, accidental fragmentation and disconnection of the entered data is prevented by the relational database design.
- Using the relational database approach, with linked independent tables containing the actual entries, it is easily possible to add or edit single tables if necessary, without redesigning the entire database. As the conceptual structure and the principles of implementation of CoCoDat are formed by experiences with the CoCoMac database of macaque brain connectivity (Stephan et al., 2001), the integration of the 2 databases would require a relatively small effort.
- We developed additional functions allowing for a user-defined detailed search and representation of the collated data for use in e.g. modelling efforts: These will be described in detail below.

2.2 Structure of CoCoDat

CoCoDat is built using the relational database approach, where the collated data are represented in a number of separate tables arranged in hierarchical relationships. Tables are cross-referenced by *primary keys*, - a combination of values that uniquely identify each record (Ullman & Widom, 1997). The database has been implemented using Microsoft® Access 2000.

The data contained in the CoCoDat database are organized in 4 major groups: *Literature, Experimental Methodology, Mapping of Recording Site* and *Experimental Findings*. These are roughly arranged in a hierarchy following 2 streams that are anchored in bibliographical data, then diverge to a group describing the experimental procedures and another describing in detail the recording site(s) before they are re-united in the tables containing the actual experimental findings in question.



Fig. 2.1: The relationship of the 4 major data groups in the CoCoDat database.

Table	Content
Literature	
Literature	ID, title, year of publication, publication type, abstract, keywords, comments and db- collator ID
Literature_Abbreviations_Journals	Predefined list of journal name abbreviations
Literature_Authors	Authors initials and last names
Literature_BookChapters	Page numbers, editors, book title, publisher, place of publishing
Literature_Books	Publisher, place of publishing
Literature_JournalArticles	Journal, volume and page numbers
Literature_LinkTable	Links between literature IDs and authors (for search purposes)
Mapping of Recording Site	
BrainMaps	Entered BrainMaps (here only General Map)
BrainMaps_BrainSites_BrainSiteTypes	Defined BrainSiteTypes
BrainMaps_BrainSiteAcronyms	BrainsiteAcronyms with their full description
BrainMaps_BrainSites	Brainsites given by a combination of Brainmap, BrainSiteAcronym and BrainSiteType
BrainMaps_BrainSites_HC	Hierarchical relations of BrainSites at each level of organisation
Neurons	RecordingSites given by their LiteratureID, BrainRegion, Layer, NeuronType &
	Compartment
Experimental Methodology	
Methods Electrophysiology	Experimental preparation, solution, recording method, stimulation method, species,
	temperature, text- and figure references, comments
Methods_Electrophysiology_Animals	Animal strain, age, sex and weight
Methods_Electrophysiology_CompConc	Pharmacological components and their concentrations
Methods_Electrophysiology_Preparations	Predefined list of experimental preparations
Methods_Electrophysiology_RecMethod	Predefined list of recording methods
Methods_Electrophysiology_SliceOrient	Slice orientation Predefined list of planes of section
Methods_Electrophysiology_SolutionType	Predefined list of solution types
Methods_Electrophysiology_Species	Predefined list of species
Experimental Findings	
Neurons_Morphology	Morphological feature name, value, reconstruction, citations, text- and figure references,
	comments
Neurons_Morphology_RecMethod	Morphological reconstruction methods
Neurons_FiringProperties	Firing pattern type, citations, text- and figure references, comments
Neurons_FiringProperties_APduration	Action potential duration
Neurons_FiringProperties_Kinput	Input resistance
Neurons FiringProperties TauM	Membrane time-constant
Neurons FiringProperties Vrest	Membrane resting notential
Neurons IonicCurrents	Current name charge-carrier peak conductance peak current reversal potential voltage
	threshold, half-activation voltage, peak voltage, citations, text- and figure references, comments
Neurons_IonicConductances	Conductance name, charge-carrier, peak conductance, voltage threshold, half-activation
	voltage, peak voltage, citations, text- and figure references, comments
Neurons_SynapticCurrents	Synapse type, peak conductance, peak current, peak potential, reversal potential, latency,
	citations, text- and figure references, comments
Neurons_Connectivity	Target BrainRegion, Layer, NeuronType and Compartment, citations, text- and figure
	references, comments
Neurons_ChargeCarrier	Predefined list of charge carriers

Table 2.1: The tables and contents of the CoCoDat database organized in the 4 major data groups.

Each of the 4 main groups contains several tables that will be discussed in detail below. All data (except content of predefined lists) are entered into the database by way of specifically designed graphical *forms*, allowing the user to type data into one or more tables of the main data group represented. These forms can also be used for simply browsing through the database and are selected from the *CoCoDat Switchboard* that also provides the selection of user-defined searches (Figure 2.2).

Enter/Browse Data	Perform Search	Print Reports
Bibliographic Data	Experimental Data	Experimental Data
Assign Author to Paper	Bibliographic Data	Bibliographic Data
Experimental Methods		Methodological Data
Experimental Data		
Mapping Data		
Hierarchy Data		

Fig. 2.2: The CoCoDat switchboard gives the user access to all form for entering data, querying the database and generating printable reports.

In many cases lists with predefined commonly occurring values (journal names, recording methods, charge carriers etc.) are used to facilitate the entering of data into the database.

2.2.1 Literature data

Entering literature data into the database is facilitated by the use of a graphical interface *form* giving direct access to the main *Literature* table, as well as several subtables containing bibliographical details. In addition to a unique ID assigned to each publication in CoCoDat, the *Literature* table contains information on the title, abstract, publication type, type of experimental data and the database collator for all articles in the database. The subtables contain exact bibliographical data according to the type of publication, as well as the authors full names and links between the identifying publication ID and the individual author names for search purposes.

The literature content of CoCoDat can be searched by selecting "Bibliographic Data" under the heading "Perform Search" on the *SwitchBoard*. Here a built-in Microsoft Access function is used to filter the content based on entries in fields corresponding to those shown above on the literature form. Search parameters are typed in or can be selected from drop-down menus that show values already entered in the database.

2.2.2 Methodological data

After recording the bibliographical data of a publication, information pertaining to the methodology of the experiments performed is recorded using a graphical form, giving access to all tables containing methodological information.

Data recorded during most electrophysiological experiments are extremely dependent on the specific experimental conditions and procedures utilized. Differences in recording methods or delivery of stimuli, composition of bathing solutions or even the temperature have large influences, and can make results from different publications seem contradictory, even if they are not. For the database user to be able to evaluate these methodological influences, detailed records are maintained of the experimental procedures related to each collated dataset.

The main table *Methods_Electrophysiology* contains information on experimental preparation, extra- and intracellular solutions, stimulation and recording procedures etc. Further subtables contain detailed information on the animals used in the experiments, plane of section for cortical slices and the concentration of every component of the experimental solutions that deviate from standard ACSF (artificial cerebro spinal fluid) solution. For each publication in the database, one or more experimental procedures with differing parameters can be entered.

2.2.3 Mapping data

In order to ensure an accurate and flexible attribution of the experimental data to the correct recording site, *BrainSite* designators are used, describing the anatomical position of the recording at the levels of *BrainRegion*, *Layer*, *NeuronType* and *NeuronCompartment*. The designators at the 4 levels of description are not precisely delineated such as e.g. the cytoarchitectonically defined cortical areas in the CoCoMac database. Rather these should be seen as general descriptors based on a generally accepted nomenclature, since precise definitions and techniques for unambiguously determining their properties are not currently available. As an example, neuron types and their properties are currently not defined in a strict manner according to specific principles, but described according to heuristic criteria, e.g. IB (intrinsically bursting) vs. RS (regular spiking) pyramidal neurons. For consistency with the concepts of CoCoMac, these prototypes are regard as components of a General Map (GM). The GM is a non-delineated hypothetical map conceived to organize these

prototypical *BrainSites* according to the generalized concepts used in descriptive neuroanatomy, since they do not refer to an individual brain, or maybe even species.

Each encountered description of a brain site in the literature is assigned a unique acronym for use in the database. These acronyms with their full textual description are listed in the *BrainMaps_BrainSiteAcronyms* table. Combining the information on brain map (in this case only the General Map), *BrainSiteType* (e.g. region, layer, neuron type or subcellular compartment) and the appropriate acronym yields the actual brain site listed in the *BrainMaps_BrainSites* table, with an ID of the form "GM-Ctx_Vis" (General Map, Visual cortex):

ID	ID_Acronyms_BrainSites	ID_BrainMap	Site_Type
GM-Ctx_Vis	Ctx_Vis	GM	Area_IsoCtx_3D
GM-Ctx_TempLobe	Ctx_TempLobe	GM	Area_IsoCtx_3D
GM-L6_IsoCtx	L6_IsoCtx	GM	Lamina_Ctx_3D
GM-C_Pyr	C_Pyr	GM	Neuron_3D
GM-Soma	Soma	GM	Neuron_Compartment_3D
GM-Dend	Dend	GM	Neuron_Compartment_3D
GM-Axon	Axon	GM	Neuron_Compartment_3D

Table 2.2: Entries in the table *BrainMaps_BrainSites* for visual cortex, temporal lobe, layer 6 in isocortex, pyramidal neuron, soma, dendrite and axon.

A way of uniquely identifying a particular site in the brain was adopted by combining 4 such *BrainSite*-IDs on the 4 levels of description: *BrainRegion, Layer, NeuronType* and *NeuronCompartment*. The four levels can be seen as a hierarchical arrangement of successive levels of detail. However, frequently a more general category is not specified so that these levels have to be regarded as independent. This view paves the way for powerful search options (see below). Combined with the *literature-ID* of the publication in question, they constitute a *RecordingSite* entry in the *Neurons* table of the form seen in table 2.3.

ID_Literature	ID_BrainRegion	ID_Layer	ID_NeuronType	ID_NeuronCompartment
B00b	GM-Ctx_SeM	GM-L5_IsoCtx	GM-C_Pyr	GM-Soma
BGC00	GM-Ctx_B	GM-L4_IsoCtx	GM-C_FS	GM-Comp_Gen
KHP96a	GM-Ctx_SS	GM-L_InfraGr_IsoCtx	GM-C_Pyr	GM-Dend_Ap
KLLB94a	GM-Ctx_Vis	GM-L5_IsoCtx	GM-C_Pyr	GM-Comp_Gen
LESF00	GM-Ctx_B	GM-L4_IsoCtx	GM-C_SpiN	GM-Axon
LESF00	GM-Ctx_B	GM-L4_IsoCtx	GM-C_SpiN	GM-Comp_Gen

Table 2.3: Entries in the *Neurons* table. The first entry describes the soma of a layer 5 pyramidal neuron from the sensory-motor cortex from Bekkers (2000).

The combination of the 5 fields provides a unique reference to a single *RecordingSite* from a particular publication. In case the publication does not provide information on one or more of the 4 levels of description, the term "general" is inserted, as seen in entries 2, 4 and 6 above, where the *Comp_Gen* is the acronym for "general/unspecified compartment".

On all 4 levels of description a number of hierarchical parent/child relationships exist between the entered brain sites. The *BrainSites* designating the *NeuronCompartment* are organized in the hierarchy shown in figure 2.3.



Fig. 2.3: The hierarchical relationship of BrainSites on the NeuronCompartment level of description.

These hierarchical relationships (along with those on the levels of *BrainRegion, Layer* and *NeuronType*) are detailed in the table *BrainMaps_BrainSites_HC*, designating which *BrainSite* is the *parent* of another on the same level of description. This information is an important part of the implemented powerful search functions that will be described later.

New *BrainSiteAcronyms*, *BrainSiteTypes* and *BrainSites* can be added by the user in a dedicated form by clicking the "Mapping Data" button under the heading "Enter/Browse Data" on the main *SwitchBoard*. For convenience, the full name corresponding to the *BrainSiteAcronym* of a *BrainSite* is displayed along with the compact designation in forms where these need to be selected (e.g. when entering experimental data or searching the database).

When new *BrainSites* are entered it is imperative that their hierarchical relations to existing *BrainSites* are simultaneously entered for use in the later described search routines. This is done via the form opened by clicking "Hierarchy Data" button under the heading "Enter/Browse Data" on the *SwitchBoard*. Here the user can establish relations or remove relations for a *BrainSite* aided by an expandable visualization of the hierarchy on the relevant level of description.

2.2.4 Experimental data

To ensure CoCoDat's usefulness for collating and organizing data from the existing literature, a comprehensive number of representative articles from the last ~15 years was surveyed, spanning a broad scope of experimental findings as well as different methods and research groups. The main insight from the survey was, as expected, that the types as well as the formats of the data reported are extremely variable.

Based on the survey, the co-occurrence of reported values from different experiments noted, resulting in a division of the experimental data into the 6 categories *Morphology, FiringProperties, IonicCurrents, IonicConductances, SynapticCurrents* and *Connectivity.* These categories, as well as the co-occurrence of reported values, reflect the experimental methodology and the underlying biophysical theory by its division of information into a set of "building blocks" that constitute and define a neuron. A cell is seen as a basic structure defined by its morphology, with substructures furnished with different sets of voltage-gated and synaptic currents/conductances, that, when combined, determine the neurons measurable "macroscopic" behaviour or *FiringProperties.* When seen as part of a larger network, determining the *Connectivity* also becomes necessary. An experiment often focuses on one of the 6 categories, however, frequently data are also reported in the categories *Morphology* and *FiringProperties* as part of a general characterization or identification of the neuron. This organization of the data nicely corresponds with the division of information and modelling objects in simulations based on the concepts of compartmental modelling (Koch & Segev, 1989; Rall, 1964).

Experimental data are entered into CoCoDat in 6 main tables (some of which contain several subtables) with fields reflecting the most commonly reported parameters in the literature. Furthermore, each entry for experimental data has a designated field for straight textual quotes for clarifying purposes, as well as the opportunity to include direct reports of data not fitting the pre-defined fields. Data are entered using custom-defined forms after selecting the appropriate *RecordingSite*. Each entry is linked to both the ID of the neuronal recording site and the experimental method in question. A different set of standard fields defined in each of the six *DataCategories*, including direct references to the text and figures containing the data, are filled with the available information. In the field *Comments* the data collator can enter comments or summarized information not fitting in any other fields. The contents of the six main tables storing experimental data is summarized by table 2.1.

2.2.5 The relational structure of the database

As mentioned above, CoCoDat is a relational database with the actual data arranged in hierarchically linked independent tables. The tables are linked by one-to-many relationships downwards in the hierarchy, meaning that e.g. a particular article can contain information on several experimental methods or recording sites that again can be associated with several experimental findings. The relational structure of CoCoDat is shown in figure 2.4.



Fig. 2.4: The relational structure of the main tables in the CoCoDat database.

Here the aforementioned division from the initial literature entry into 2 streams of methodological and mapping data, respectively, that are re-united at the level of the 6 experimental data categories is easily recognizable.

2.2.6 Current content of CoCoDat

At present most of the collation has been targeted on data necessary for modelling of local circuitry in the barrel cortex of the rat, with a primary focus on single cell properties of layer 5 pyramidal neurons (see below). The current contents stems from 37 publications with 76 records on experimental methodology and 65 neuronal recording sites. Linked to these are a total of 208 records of experimental data distributed as: *Morphology* (42 records), *FiringProperties* (52 records), *IonicCurrents* (68 records), *IonicConductances* (15 records), *SynapticCurrents* (11 records) and *Connectivity* (20 records).

2.3 Extracting and representing datasets

Datasets are conveniently extracted from CoCoDat using the *SearchBoard*, which is selected from the main *SwitchBoard* of the database (Figure 2.2). Here the user specifies the constraints on the neuronal structure(s), type of data and animal to be included in the search by selecting the desired *BrainRegion*, *Layer*, *NeuronType* and *NeuronCompartment* along with *DataCategory* and *Species* (specifying species is optional). All search terms are combined to a final search string by use of the Boolean AND operator.

Fig. 2.5: The CoCoDat *SearchBoard*. Search criteria are selected using the 6 drop down menus available at the top of the form.

Since data is entered in the database according to the originally specified *RecordingSite* in a publication, a simple search for data linked to the combination of the 4 selected *BrainSites* would fail to produce all relevant data: Selecting e.g. *GM-Ctx_SeM* (sensorimotor cortex) as *BrainRegion* would only return data from *RecordingSites* containing the exact matching *BrainSite* acronym, but not from substructures like e.g. *GM-Ctx_SS* (somatosensory cortex). Therefore the CoCoDat search routine first expands each selected *BrainSite* into a list where all children, as well as grandchildren etc., are

contained according to the hierarchical parent-child relationships recorded in the table *BrainMaps_BrainSites_HC*. As this is done for all 4 levels of description, the final number of *RecordingSites* to be searched is the all-to-all combinations (i.e. the Cartesian product) of the expansions of the 4 originally selected *BrainSites* (Figure 2.6).

Selected BrainSites	GM-Ctx_SS	GM-L_InfraGr_IsoCtx	GM-C_SpiN	GM-Soma
	\downarrow	\downarrow	\downarrow	\downarrow
Expanded lists			GM-C_SpiN	
	GM-Ctx_SS	GM-L_InfraGr_IsoCtx	GM-C_Pyr	
	GM-Ctx_B	GM-L5_IsoCtx	GM-C_PyrStar	GM-Soma
		GM-L6_IsoCtx	GM-C_RS	
			GM-SpiSt	
	\downarrow	\downarrow	\downarrow	\downarrow
Queried	GM-Ctx_SS	GM-L_InfraGr_IsoCtx	GM-C_SpiN	GM-Soma
Combinations	GM-Ctx_SS	GM-L_InfraGr_IsoCtx	GM-C_Pyr	GM-Soma
	GM-Ctx_SS	GM-L_InfraGr_IsoCtx	GM-C_PyrStar	GM-Soma
	÷	:	:	÷
	GM-Ctx_SS	GM-L5_IsoCtx	GM-C_SpiN	GM-Soma
GM-Ctx_SS		GM-L5_IsoCtx	GM-C_Pyr	GM-Soma
	:			:
	GM-Ctx_B	GM-L6_IsoCtx	GM-C_SpiSt	GM-Soma

Fig. 2.6: The expansion and combination of the four original *BrainSites* somatosensory cortex (GM-Ctx_SS), infragranular layers of isocortex (GM-L_InfraGr_IsoCtx), spiny neurons (GM-C_SpiN) and soma compartment (GM-Soma). Arrows represent the different steps in the expansion and combination of the entered search terms and dotted lines signify compressed entries in the list of queried combinations. A total of 30 hypothetical *RecordingSites* are submitted for the query after the all-to-all combination.

From this it also follows that by selecting the topmost (general) *BrainSite* at each level (*GM-Ctx_Gen, GM-L_Gen, GM-C_Gen* and *GM-Comp_Gen*), the user can extract all information in a *DataCategory* for a given *Species* currently contained in CoCoDat.

To extract the desired experimental data, the hypothetical neuronal structures generated by the all-to-all combinations are first checked for existence by searching the *RecordingSites* table, and the IDs of those found are then used to filter the entries in the selected *DataCategory*. The resulting records are again filtered according to the specified *Species* by using the link to the *Methods Electrophysiology* table, where the species is recorded.

Since it is often the case that the queried information for a particular *RecordingSite* is not available, either because it has not been entered in CoCoDat yet or because it has never been reported in the literature, a feature allowing the user to automatically relax the search-criteria was implemented. By choosing to automatically relax the criteria specifying one of the four levels of description, one can allow the search routine to select the parent of the initially selected *BrainSite* and repeat the procedure with a new set of combinatorically generated *RecordingSites*, if the original search does not provide any results. This provides the user with the choice of selecting the level of description he or she deems to be the least

critical in terms of defining the neuron to be modelled, which may then produce usable results from a similar recording site, that can be used directly or modified to fit the model in question.



Fig. 2.7: The 4 tree-view windows displaying the *BrainSites* constituting the *RecordingSites* with the desired search results.

To allow for a direct visual evaluation of the extracted data, a hierarchical representation of the *RecordingSites* based on the hierarchy of each *BrainSite* involved is generated. A branching-tree structure visualizing the hierarchy based on the relationships defined in *BrainMaps_BrainSites_HC* is then displayed in the 4 panels with a number in parentheses giving the number of returned results for a given *BrainSite* (Figure 2.7). Here the user can directly determine the distribution of returned information among the selected *BrainSites*. In the case where information is not available on the structure to be modelled and related *BrainSites* have been included in the search, the user can furthermore visually appraise the "logical" distance between the *RecordingSites* of returned results and the modelled structure, which will aid in determining which results are most appropriate for the modelling.

By clicking the "Show Details" button under the "Perform Action" heading, the user can call up a form summarizing the returned search results (Figure 2.8).

3 SearchResults : IonicConductances									
		NeuronID	ID_BrainRegion	ID_Layer	ID_NeuronType	ID_NeuronCompartment	Conductance_n	Charge_carrier	PrintResult
	+	998919415	GM-Ctx_SeM	GM-L_InfraGr_IsoCt	GM-C_Pyr	GM-Comp_Gen	BK	K+	V
	+	-625879160	GM-Ctx_SeM	GM-L5_IsoCtx	GM-C_Pyr	GM-Soma	I(A)	K+	V
	+	-625879160	GM-Ctx_SeM	GM-L5_IsoCtx	GM-C_Pyr	GM-Soma	I(K)	K+	V
	+	1927746281	GM-Ctx_SeM	GM-L5_IsoCtx	GM-C_Pyr	GM-Dend_Ap	I(A)	K+	V
	+	-1208544038	GM-Ctx SS	GM-L InfraGr IsoCt	GM-C Pyr	GM-Comp Gen	BK	K+	V
	+	-1208544038	GM-Ctx SS	GM-L InfraGr IsoCt	GM-C Pyr	GM-Comp Gen	BK	K+	
	+	-1208544038	GM-Ctx SS	GM-L InfraGr IsoCt	GM-C Pyr	GM-Comp Gen	BK	K+	
	+	-945125805	GM-Ctx SS	GM-L InfraGr IsoCt	GM-C Pyr	GM-Soma	BK	K+	
	+	-2090324328	GM-Ctx SS	GM-L InfraGr IsoCt	GM-C Pyr	GM-Dend Ap	BK	K+	
	+	1006790213	GM-Ctx SS	GM-L5 IsoCtx	GM-C Pyr	GM-Soma	BK	K+	
	+	1006790213	GM-Ctx SS	GM-L5 IsoCtx	GM-C Pyr	GM-Soma	K(fast inact.)	K+	
	+	1006790213	GM-Ctx SS	GM-L5 IsoCtx	GM-C Pyr	GM-Soma	K(slow inact.)	K+	
	÷.	1006790213	GM-Ctx SS	GM-L5 IsoCtx	GM-C Pyr	GM-Soma	K(very slow ina	K+	
	+	1006932736	GM-Ctx_SS	GM-L5 IsoCtx	GM-C Pyr	GM-Dend Ap	BK	K+	
•	+	1006932736	GM-Ctx SS	GM-L5 IsoCtx	GM-C Pyr	GM-Dend Ap	К	K+	
*						a a construction of the state o		72/18	

Fig. 2.8: The summary form gives an overview of the returned search results.

Here the 4 *BrainSites* constituting the *RecordingSite* are displayed, along with the basic qualitative information for the data, i.e. type of ionic current, morphological feature described etc.. By clicking the "+" signs next to the entries, a form containing all detailed information for the selected *RecordingSite* (each given by the associated *NeuronID*) is expanded and the user can navigate between the entries using the arrows of the forms navigation bar (Figure 2.9).



Fig. 2.9: Detailed view of one expanded entry in the summarized list. The insert shows the pop-up window with information on the same recording site in the *IonicCurrents* data category.

By clicking "Literature" on a particular form containing experimental findings, the user brings up the bibliographical information available on the publication in a separate window. Similarly, a separate window detailing the experimental procedures used can be opened by clicking "Experimental Methods". To check the content of other *DataCategories* for the displayed *RecordingSite* in a given study, the user can click one of five buttons linking to pop-up windows displaying the desired information.

After inspecting the detailed experimental information returned by the search routine on the individual *RecordingSites*, the user can select the most appropriate information to be printed in *Report* form by returning to the *SummaryForm* (Figure 2.8) and activating the tick boxes of desired entries in the rightmost column. When returning to the main *SwitchBoard* (Figure 2.2), reports of the experimental data with reference to the *RecordingSite*, as well as the associated bibliographic and methodological information (Figure 2.10), can be generated for printing by clicking one of three buttons under the heading "Print Reports".

NeuronID	-625879160	Conductance_name	I(A)
ID_Literature	В00ь	Charge_carrier	K+
ID_Methods_Electrophysiology	1006622979	Peak_conductance	8.5 +- 0.3 pS
		V_threshold	171
		V_half_activation	-24.5 +- 3.7 mV
ID_BrainRegion ID_Layer	GM-Ctx_SeM GM-L5_IsoCtx	V_peak	6-6
ID_Neuron Type ID_Neuron Compartment	GM-C_Pyr GM-Soma	Reference_text	pp.613-614
Citations		Reference_figures	Fig. 2
"Two distinct types of single-chann shorter open times and a mean cond (Fig. 2A)." p.613	el were observed in cell-attached and ou fuctance of 8.5 +- 0.3 pS (+- S.E.M., n=	ntside-out somatic patches (Figs 2 3; Fig. 2). Averages of this channe	and 3). One type of channel had el type gave a pure I(A)-like current
"For convenience, the fast transient respectively."p.614	and slowly inactivating/non-inactivatin	g currents seen in these patches w	ill henceforth be called $I(A)$ and $I(K)$,
Comments			
More parameters on activation/inac	tivation and timeconstants given.		
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Figure 2.10: An example of a printable report with data from the *IonicConductance* data category.

2.4 Distributing CoCoDat

The CoCoDat database can be downloaded as a database file for Microsoft Access from http://www.cocomac.org along with a manual detailing procedures for entering and extracting datasets. CoCoDat is copy-lefted under the GNU General Public License to promote further use and distribution. Since all data extracted from the published literature contained in the database are quoted with full references and do not include reproductions, the distribution of these do not constitute a copyright problem.

2.5 Summary

The CoCoDat database provides a powerful and flexible environment for organizing detailed records of quantitative experimental data for biophysically realistic modelling, with a data-grouping according to electrophysiological methodology and modelling practice that facilitates the extraction of data for a specific part of a model. Additional detailed information on experimental methodology eases evaluation or reconciliation of apparent contradictions in the published experimental data, while detailed references to

textual and graphical information allows the user to rapidly locate relevant material in the original publications.

The implementation of a hierarchically organized mapping scheme covering 4 levels of description (BrainRegion, Layer, NeuronType, NeuronCompartment) allows for the extraction of subcompartment specific information useful for constructing neuron models with differentially distributed membrane conductances and passive parameters. The use of a hierarchically arranged BrainSites in the mapping scheme furthermore allows for a flexible extraction of data from the database, using a combinatorical search-algorithm to deliver results matching the user specified criteria. By invoking the optional automatic relaxation of the initially specified search-criteria on the level of description deemed least important to a given model, the user can extract the closest possible matching datasets from the database in cases where information on a specifically requested *RecordingSite* is not available. By manually varying the search criteria once a useful dataset has been found, the user can perform a thorough exploration of the surrounding dataspace. Using the implemented functions to generate printable date-stamped reports of selected datasets, as well as their related data in other data categories and associated methodological and bibliographical information, makes it possible to easily file and track the information available in the database at the time a given model was constructed.

Database	Bibliographic Data	Methodological Data	Experimental Data	Model data	Search Functions	Online Accessibility	Additional
CoCoDat ¹	Detailed	Detailed	Quantitative, uniterpreted data on morphology, firing properties, ionic currents and conductances, synaptic currents and connectivity	Quantitative	Advanced combinatorial searches and hierarchical representations	Downloadable	Can be integrated with the CoCoMac database
Duke / Southampton Archive of Neuronal Morphology ² (Cannon et al., 1998)	References	Some	Complete downloadable experimentally obtained morphologies, few electrophysiological data	None	Selectable from lists	Yes	Software for viewing, editing and converting morphology files. Images of cells.
Virtual Neuromorphology Electronic Database ³ (Ascoli et al., 2001)	References	Some	Complete downloadable experimentally obtained and algorithmically generated morphologies	None	Selectable from lists	Yes	Software for viewing, editing and converting morphology files. Images of cells.
SenseLab Databases (CellPropDB, NeuronDB, ModelDB) ⁴ (Mirsky et al., 1998)	References	Some	Qualitative	Downloadable implementations of contributed models	Selectable from lists and some combinatorial search functions	Yes	Integrating information on genes and expressed proteins
Hippocampus neuron data files (Gonzalez et al., 2001)	References	Detailed	Complete downloadable experimentally obtained morphologies	None	Selectable from lists	Yes	Images of cells.

 Table 2.4: Comparison of different databases containing data on the single neuron and microcircuitry level.

 (¹http://www.cocomac.org, ²http://www.cns.soton.ac.uk/~jchad/cellArchive/cellArchive.html, ³http://www.krasnow.gmu.edu/L-Neuron/database/index.html, ⁴http://senselab.med.yale.edu/senselab/, ⁵http://cascade.utsa.edu/bjclab/)

As can be seen in table 2.4, the CoCoDat database provides the modeller with resources not previously available from existing databases at the microcircuitry and single neuron level, by supplying un-interpreted detailed quantitative data of all types along with the detailed methodological information important for assessing whether a set of data is appropriate for a use in a given modelling study. Existing models can be downloaded for use from ModelDB (Mirsky et al., 1998). This is however only useful if the scientist is looking for a general model of a neuron type or if the model code is sufficiently modular to facilitate easy integration of e.g. a new morphology for modelling of a specific cell. Even so, a certain amount of time must be devoted to adjusting the distributions and kinetics of voltage-gated conductances to the new morphology and possible differences in experimental conditions.

The use of CoCoDat is not limited to the amount of information contained in the centrally downloadable version of CoCoDat: The user can continuously increase his or her personal data collection using the appropriate forms selectecable from the central *SwitchBoard* (Figure 2.2). Detailed guidelines for collating and entering data in all categories is provided in the CoCoDat manual, as well as instructions for adding *BrainSites* to the already established hierarchies. For a group of researchers involved in modelling similar neuronal structures, setting up a central master database with more database-clients for entering new data, would allow for the rapid accumulation of a large amount of centrally accessible data for current and future modelling studies. In situation where more collators

are entering data into the same database, it is highly advisable that procedures for proofreading records are established to ensure common standards are being adhered to, as has been established for the CoCoMac database (Stephan et al., 2001).

The CoCoDat database shares the principal structure of the connectivity database CoCoMac (Stephan et al., 2001), and due to compatible mapping schemes the information in the two databases could easily be integrated, providing a the opportunity to link detailed information large scale anatomical connectivity and local microcircuitry properties, giving a more complete picture of the nature and impact of networks in the brain. Due to the modular nature of the database, it is furthermore straightforward to add additional data categories in order to incorporate information on e.g. intracellular signaling or distributions of ligand or voltage-gated ion channels as reported from immunohistochemical studies. Alternatively information from other existing databases could be linked to *RecordingSites* in CoCoDat using the XML capabilities of Microsoft Access.

3 Implementation of a detailed layer V IB pyramidal neuron model

Using models to investigate biological systems allows the scientist to investigate the interplay and importance of many parameters and parts of a system for various behaviours, which is often impossible to do simultaneously in the experimental situation. This allows for detailed analysis of already observed phenomena and possibly predictions that can subsequently be tested in the laboratory.

Modelling in neuroscience generally falls under one of two complementary paradigms: *Top-down* and *Bottom-up*. Top-down modelling starts from systems properties and mostly uses populations of relatively abstract units connected either randomly or in all-to-all fashion to investigate how networks can accomplish certain tasks known to be handled by nervous systems, such as feature extraction, binding or synchronized responses. Evidence for a similarity between the features of the abstract model and the actual biological system are subsequently sought, and the models can then be modified to give better results, - typically by adjusting connectivity patterns or the weight of classes of connections but not the simplified representations of individual units.

Bottom-up modelling takes the opposite approach and synthesizes complex models of individual neurons or neuronal circuits by combining experimentally reported data on neuronal features such as detailed morphologies, distributions of ionic channels, characteristics of synaptic inputs etc. The parameters describing all these characteristics are then tuned until the response of the model to simulated stimuli fit the responses reported from experimental studies.

3.1 Compartmental modelling

The modelling approach used in this thesis is the *bottom-up* approach, which has evolved from early application of Lord Kelvin's equations describing the first trans-atlantic telegraph cable. This was applied to the investigation of nerve processes, axons and dendrites, idealized as cylinders with a conductive core (the intracellular cytoplasm) isolated by from an iso-potential surrounding medium by the dielectric cell membrane. Using the partial differential equations of Lord Kelvin, the development of signals in such

a nerve cylinder after a current injection and the influence of passive parameters like membrane resistivity (R_m) and capacity (C_m) as well as cytoplasmic (or *axial*) resistivity (R_i) was characterized under different conditions. A common formulation of the cable equation is

$$\lambda^2 \frac{\partial^2 V_m}{\partial x^2} - \tau_m \frac{\partial V_m}{\partial t} - V_m = 0$$

where V_m is the transmembrane potential, the time constant $\tau_m = R_m C_m$ and length constant $\lambda = \sqrt{(R_m/R_i)(d/4)}$ (with *d* the cylinder diameter, R_m and R_i the specific membrane resistance and intracellular resistivity respectively and C_m the membrane capacity). In solutions to the cable equation the time and length constants govern the decay of potential changes over time or distance. A large τ (which depends only on specific membrane capacitance and resistance) means that the signal decays slowly, and thus provides a large time window for integrating temporally separated inputs. Similarly a large λ (which furthermore depends on the diameter of the cylinder) allows for integration of spatially distributed input as the attenuation with distance will be small.

When Hodgkin and Huxley's (1952) groundbreaking work established that current flow over the neuronal membrane during action potentials occurred through selective conductances controlled by the transmembrane voltage, a new modelling approach was needed, since the fundamental assumptions in cable theory (uniformity of membrane and cytoplasm, time-independent membrane parameters) were no longer valid and the differential equations thus cannot be solved analytically.

Willfred Rall (1964) introduced the concept of compartmental modelling in which the membrane cylinders are discretized into smaller interconnected homogenous pieces. When each of these *compartments* is small enough to be considered isopotential within each timestep of a simulation, the problem reduces to a number of coupled ordinary differential equations, which can be solved numerically. Below, the concepts of compartmental modelling will be discussed in detail, including the modelling of voltage-gated ionic conductances and synaptic conductances based on (Nelson & Rinzel, 1998; Segev, 1998; Koch, 1999; Segev and Burke, 1998).

3.1.1 The compartmental description

The basic concept of compartmental modelling is the discretization of the neuronal morphology in to small homogenous patches of membrane. These must be small enough to be considered isopotential along an axis taken parallel to the membrane, since this means that the transmembrane potential (with the extracellular medium taken as an isopotential zero or ground reference) will not vary across the piece of membrane described. If we consider a piece of a dendrite, and assume rotational symmetry (i.e. homogenous distribution of active and passive properties in the membrane) such an idealized cylindric compartment can be described by an equivalent circuit diagram as seen below:



Fig. 3.1: A homogenous piece of neuronal membrane idealized as a cylindric compartment *j* can be represented by an equivalent electric circuit with representation of constant leak conductance $(g_{leak,j})$, variable synaptic $(g_{syn,j})$ and voltage-gated $(g_{act,j})$ conductances and a capacitance representing the dielectric characteristics of the lipid bilayer (c_m) .

The membrane segment is represented as four branches in parallel representing the constant leak conductance g_{leak} , the synaptically activated conductances g_{syn} , the voltagegated conductances g_{act} and the membrane capacitance c_m . Each conductance is coupled in series with a battery (or electromotive force) symbolizing the electrochemical Nernstpotential of the ions the represented channel is permissive to

$$E_{ion} = (RT/zF)\ln(C_{out}/C_{in})$$

where *R* is the gas constant, *T* the temperature, *z* the valence of the ion, *F* is Faradays constant and *C* the concentration of the ion outside and inside the cell respectively. This equilibrium potential is often referred to as the reversal potential E_{rev} of the conductance, since the direction of the current flow (into or out of the cell) will change when the membrane potential crosses the reversal potential. In cases where a current is carried by
more than one type of ion, the reversal potential is the weighted average of the Nernstpotentials of all permeating ions, determined by their relative permeability.

As can be seen in figure 3.1, the extracellular space is taken as ground or zero potential, which means that the typical resting potential across the neuronal membrane is negative. As all components are coupled in parallel, the total current crossing the membrane is the sum of currents through the ionic conductances and the displacement current charging the capacitance

$$I_{m,j} = c_{m,j} (dV/dt) + g_{leak,j} (V_j - E_{leak,j}) + g_{v-gated,j} (V_j - E_{v-gated,j}) + g_{syn,j} (V_j - E_{syn,j})$$

Most models consist of more than one compartment, with the simplest case being a linear chain of compartments.



Fig. 3.2: A chain of compartments j-1, j and j+1 connected by axial conductances.

According to Kirchoffs current law, the sum of all currents to and from a branch-point in a circuit must be zero, which in this case means the current passing the membrane in compartment *j* must be equal to the difference between the currents running axially from *j* to j-1 and *j* to j+1

$$I_{m,j} = g_{j-1,j} (V_{j-1} - V_j) - g_{j,j+1} (V_j - V_{j+1})$$

Combining the two expressions for $I_{m,j}$ and isolating the capacitive current leads to an ordinary differential equation for the potential across the membrane of a compartment, which for each compartment in a model can be solved by numerical integration

$$c_{m,i}(dV_{i}/dt) = g_{i-1,i}(V_{i-1} - V_{i}) - g_{i,i+1}(V_{i} - V_{i+1}) - g_{leak,i}(V_{i} - E_{leak,i}) - g_{v-gated,i}(V_{i} - E_{v-gated,i}) - g_{syn,i}(V_{i} - E_{syn,i})$$

This description is not limited to the case of compartments connected in a chain, but can involve any number of compartments at a branchpoint. In that case more contributions must simply be added to account for the extra axial currents.

3.1.2 The Hodgkin-Huxley formalism for voltage-gated conductances

The time- and voltage-dependent conductance changes of channel proteins in the neuronal membrane is modelled using the Hodgkin-Huxley formalism initially developed for the sodium and potassium conductances underlying the action potential in the squid giant axon (Hodgkin & Huxley, 1952). Whereas it is well established that ionic channels can enter several substates (Hille, 1992), the original Hodgkin-Huxley model with only *open* or *closed* states is still the preferred description for neuronal modelling, unless the modeller is interested in the detailed behaviour of a specific ionic channel.

The basic hypothesis was that the conductance state of each ionic channel is controlled by a number of voltage sensitive particles or *gates*, which depending on the transmembrane potential can be in either a permissive or non-permissive state for current flow through the channels. A gate that at the resting membrane potential is in a non-permissive state and opens when the potential is perturbed is termed an *activation* gate, whereas an initially permissive gate which closes after potential changes is termed an *inactivation* gate. For a population of voltage-gated ionic channels the instantaneous conductance g will then be

$$g = g_{\max} m^x h^y$$

where g_{max} is the maximal conductance when all channels are open, *m* and *h* the fraction of activation and inactivation gates being in permissive states and *x* and *y* the number of respectively activation and inactivation gates for the channel. For an activation gate assumed to follow first-order kinetics the scheme for transitions between *1-m* non-permissive and *m* permissive states is

$$1 - m \xrightarrow{\alpha_m \atop \beta_m} m$$

where α_m and β_m are voltage-dependent rate constants. The kinetics follow the differential equation

$$\partial m/\partial t = \alpha_m(V)(1-m) - \beta_m(V)m = (m_\infty(V) - m)/\tau_m(V)$$

where the steady-state fraction $m_{\infty}(V)$ and time constant for transition between nonpermissive and permissive states $\tau_m(V)$ are given by

$$m_{\infty}(V) = \frac{\alpha_m(V)}{\alpha_m(V) + \beta_m(V)} \qquad \tau_m = \frac{1}{\alpha_m(V) + \beta_m(V)}$$

The steady-state activation and time constant as function of the membrane potential is determined experimentally using a *voltage-clamp* protocol, where the membrane potential of a neuron is stepped abruptly from a holding potential V_h to a clamp potential V_c , - typically while pharmacologically blocking other conductances. Here the activation will reach the steady-state value $m_{\infty}(V_c)$ from $m_{\infty}(V_h)$ following an exponential time course

$$m(t) = m_{\infty}(V_c) - (m_{\infty}(V_c) - m_{\infty}(V_h))e^{-t/\tau_m}$$

Systematically clamping the neuron to different potentials will provide tabulated values of m_{∞} and τ_m as a function of the membrane potential. Since the gates are presumed to be independent and their state only dependent on the transmembrane voltage, the steady-state ratio of open (O) to closed (C) gates in a channel population can be described using a Boltzmann distribution with the ratio as a function of the energy change due to the movement of the gating charge

$$\frac{O}{C} = \exp\left(-\frac{w - z_g eV}{kT}\right)$$

where w is the change in conformational energy by opening the gate with zero membrane potential, z_g the gating charge, e the elementary charge and V the transmembrane potential difference. Rewriting this as the steady-state fraction of open gates at the transmembrane potential V one gets

$$\frac{O}{O+C} = \frac{1}{1 + \exp((w - z_g eV)/kT)}$$

Since this expression depends on several unknowns (such as the exact gating charge, distance moved by the gating charge and the conformational energy of the channel protein), it is not possible to directly calculate the steady-state fraction of open gates, but it can be used to fit the experimentally obtained values of m_{∞} to an expression involving the so-called half-activation voltage $V_{1/2}$ and an empirically determined slope-factor (often termed *k*) of the form

$$m_{\infty} = \frac{1}{1 + \exp((V - V_{\frac{1}{2}})/k)}$$

Using either fitted expressions for m_{∞} and τ_m or simply the tabulated experimental values, the rate constants α_m and β_m can be obtained using

$$\alpha_m(V) = \frac{m_{\infty}(V)}{\tau_m(V)} \qquad \beta_m(V) = \frac{1 - m_{\infty}(V)}{\tau_m(V)}$$

which again can be fitted to functions of typically either linoid, sigmoid or exponential shape. For channels also equipped with inactivation gates, similar expression must be calculated for the inactivation gate typically designated *h*. Generally it is not possible to *a priori* determine the number of gates for a voltage-gated conductance, thus this is done as part of the fitting procedure.

3.1.3 Synaptically activated conductances

Synaptic channels are membrane proteins chemically gated by a neurotransmitter released from the axon-terminal of a presynaptic neuron following an actionpotential. The transmitter diffuses across the synaptic cleft and binds to the receptor(s) linked to the channel, causing a conformational change that makes diffusion of the charge-carrier possible. Subsequent closing of the channel follows from inactivation of the channel, dissociation of the transmitter from the receptor and re-uptake of the transmitter by the presynaptic neuron. The time-course of the conductance change following activation of a population of synaptic channels in a compartment by single action potential is generally modelled by a dual exponential function

$$g_{j,syn}(t) = \frac{Ag_{\max}}{\tau_1 - \tau_2} \left(e^{-t/\tau_1} - e^{-t/\tau_2} \right)$$

where g_{max} is the maximal conductance when the entire channel population is open, A is a normalization constant and τ_1 and τ_2 are the time constants of activation and inactivation. These constants must be fitted experimentally to data obtained for the synapse in question.

More advanced models of synaptic channel kinetics allowing for incorporation of phenomena like receptor desensitization, priming and saturation have been implemented using Markov chain kinetics (Destexhe et al., 1998), but are primarily used when studying very detailed behaviour at the single synapse level.

3.1.4 Model implementations

Compartmental neuronal models are primarily either written up as custom programs in generic programming languages (Douglas & Martin, 1991; Traub et al., 2003) or implemented in a neuronal simulation environment like Neuron or GENESIS (De Schutter

& Bower, 1994; Mainen et. al, 1995; Stuart & Spruston, 1998), with either approach having its own advantages. Custom written simulations are generally faster, as they need only contain the exact code necessary to run a given simulation, and can be modified to include any detail desired for investigating a particular question. Implementing models in a dedicated simulation system provides the user with an environment tailored particularly for neuronal modelling, including utilities like visualization tools and input-output functions ready to use. These extra features do however often slow down the simulation itself, since the code supporting all extra features will be running regardless whether these are used or not. Extending the underlying code in an existing simulation system to include new features can be tedious, as all new components must adhere to the general structure of the simulation environment.

In this thesis, models were implemented in GENESIS (GEneral NEural SImulation System) (Bower & Beeman, 1998), which represents entities like compartments, voltagegated and synaptic channels as hierarchically arranged objects communicating via messages. GENESIS additionally provides a large array of graphics objects for visualization, functions for setting up and running simulations and additional objects necessary for simulating realistic "experiments" like e.g. pulse generators.

3.2 A detailed model of a layer V intrinsically bursting pyramidal neuron

The NeuroLucida reconstruction of a biocytin filled intrinsically bursting (IB) layer V pyramidal neuron from the rat barrel cortex characterized in slice-experiments (Schubert et. al, 2001) was selected for model implementation using the previously described principles of compartmental modelling.



Fig. 3.3: A. The experimentally recorded IV-characteristics of the IB layer V pyramidal neuron selected for modelling. B. The scaled and edited IB neuron morphology with distinctions between apical subdivisions indicated. Following the central and leftmost branches of the medial apical dendrite, the distal part is taken to start at the first branch point before the apical tuft.

3.2.1 Morphology

А

The reconstructed neuron morphology was edited for modelling using the CVAPP software package (Cannon et. al, 1998). The morphology was divided into subdomains of *soma*, *basal* and *apical* dendrites, with the apical dendrite further subdivided into *proximal*, *medial* and *distal* parts (Figure 3.3) and the part of apical dendrite prior to the first oblique apical branches was designated as the *apical shaft*. The original reconstruction of the somatic outline was replaced by a single spherical compartment with a diameter of 20 μ m, corresponding to a somatic surface of 1256 μ m², similar to sizes reported from 3D reconstructions of large layer V pyramidal neurons (White et. al, 1994).

The morphology was corrected for shrinkage during tissue processing procedures by scaling all dimensions by a factor of 1.15, obtained for the x and y dimensions by matching the reconstructed morphology to a high resolution photomicrograph of the

recorded neuron in the original slice. It has been reported that tissue shrinkage in the z direction (depth of the slice) is not necessarily comparable to actual linear shrinkage of the dendrites in this direction (Jaeger, 2001). Therefore the *x-y* scaling factor was also applied in the *z*-direction to avoid artificial elongation of the dendrites. Finally the reconstructed morphology was aligned to a photomicrograph of the biocytin-filled original neuron by rotating it to within a 0.5° match (rotation around the soma coordinate).

The compartment sizes obtained during the NeuroLucida reconstruction of a neuronal morphology are not guaranteed to satisfy the criterion for isopotentiality necessary to obtain stable solutions in the simulations. Consequently, the compartment sizes of the edited morphology were recalculated using CVAPPs built-in meshing function with default parameters ($R_m = 5 \ \Omega m^2$, $R_i = 1 \ \Omega m^{-1}$, maximal compartment length = 0.01 λ) resulting in a total of 2056 compartments. This edited morphology was exported to GENESIS celldescriptor-file format, where a correction for the area of missing dendritic spines was included by adding 0.83 μm^2 surface area per linear μm of length to dendritic compartments (Mainen & Sejnowski, 1996).

Information retrieved from the CoCoDat database (Table 3.1) was used to equip the morphology with passive parameters and active conductances, using the following search parameters for the *RecordingSite*: *GM-Ctx_SeM* (sensorimotor cortex), *GM-L_5* (layer V), *GM-C_Pyr* (pyramidal cell) and *GM-Comp_Gen* (general compartment).

Neuronal Compartment	Search results
General compartment	IK, INAF, INAP and ICa (Mantegazza et al., 1998), distribution of passive membrane parameters and Ih
	(Stuart & Spruston, 1998)
Soma	Fast inactivating I _A /g _A and slowly or very slowly inactivating I _K /g _K (Bekkers, 2000), fast inactivating
	I_{K}/g_{K} and slowly or very slowly inactivating I_{K}/g_{K} , calcium-ion dependent BK (Korngreen & Sakmann,
	2000) , I _h (Williams & Stuart, 2000)
Basal dendrites	I_{Ca} (Oakley et al., 2001)
Apical dendrite	I_{Ca} (Oakley et al., 2001), Fast inactivating I_A/g_A (Bekkers, 2000), fast inactivating g_K and slowly or very
	slowly inactivating g_K , calcium-ion dependent BK (Korngreen & Sakmann, 2000), I_h (Williams &
	Stuart, 2000)
Distal apical dendrite	I _{Ca} and I _{Na} (Schiller et al., 1997)

Table 3.1: Search results for different membrane domains from the CoCoDat database.

3.2.2 Passive membrane parameters

The passive membrane parameters were fitted using the experimentally recorded responses to a series of 200 ms hyperpolarizing current pulses injected in the neuron soma in wholecell patch configuration under current-clamp conditions (Schubert et al., 2001). As the IB neuron displays a prominent sag in membrane potential during strongly hyperpolarizing current injections, the kinetics and conductance density of a hyperpolarization-activated mixed cation conductance g_h were fitted simultaneously (see below).

Specific membrane resistance has been reported to be non-uniformly distributed in the soma and dendrites of layer V pyramidal neurons from the rat somatosensory cortex (Stuart and Spruston, 1998), following an expression of the form

$$R_m = R_{m,distal} + \frac{R_{m,soma} - R_{m,distal}}{1 + e^{(500 \mu m - dist)/-50 \mu m}}$$

where *dist* is the linear distance from the soma to a given compartment in the morphology. Specific intracellular resistance R_i and membrane capacity C_m were assumed to be constant throughout the neuronal morphology. The model responses to simulated 200 ms hyperpolarizing somatic current injections were fitted to the recorded experimental data by manually adjusting the passive electrical parameters to achieve the best possible match (Table 3.2).

R _i	C _m	R _{m,soma}	R _{m,distal}	Eleak
$0.9 \ \Omega m^{-1}$	0.007 Fm ⁻²	$4.0 \ \Omega m^2$	$0.27 \ \Omega m^2$	-0.08 V

Table 3.2: Fitted passive parameters of the neuronal model.

The resulting passive parameters are in the range of previously reported values used for neuronal modelling studies of pyramidal neurons (Mainen et al., 1995; Mainen & Sejnowski, 1996; Rapp et al., 1996; Stuart & Spruston, 1998).

To ensure isopotentiality, the electrotonic length of individual compartments in a model with active conductances should be in the range of 0.01λ to 0.05λ (De Schutter & Bower, 1994a; Jaeger, 2001). Figure 3.4 shows that this condition still is still fulfilled after applying the distribution of specific membrane resistances.



Fig. 3.4: A. The distribution of specific membrane resistivities R_m in the neuronal morphology. B: The electrotonic length (measured in units of the length constant λ) of individual compartments in the edited and remeshed neuronal morphology using distributed R_m values. Compartments with a relatively large electrotonic length are located in the apical tuft where the specific membrane resistance is low

As can be seen in figure 3.4, the distribution function effectively separates the modelled neuronal morphology in two domains with high (perisomatic) and low (distal) specific membrane resistivity.

3.2.3 Voltage-gated conductances

Due to the experimental difficulties of recording from the thin and extensively branched basal and distal apical dendrites, most information in the literature is reported from experiments in either whole cell configuration or using patches of somatic or apical dendritic membrane (Table 3.1). Therefore all dendritic domains were initially assumed to contain most active conductances reported for the whole cell and their regional densities adjusted during the fitting procedure.

As information on kinetics and densities of the reported conductances and currents is often incomplete (i.e. no information on time constants accompanying an expression for steadystate activation), a set of conductances used to model bursting properties of layer II/III neocortical neurons (Traub et al., 2003) was used as a basis and modified to fit the model responses to the experimental data, in combination with a detailed kinetic description of hyperpolarization-activated mixed cation conductance g_h (Williams & Stuart, 2000).

3.2.3.1 Sodium conductances

The equations describing the kinetics of the perstistent sodium conductance g_{NaP} were shifted 13 mV positive to match the excitability of the IB neuron at the resting membrane

potential. The conductance density of g_{NaP} was modelled as proportional to the density of the fast inactivating sodium conductance g_{NaF} , as experiments have shown these are likely two conducting substates of one single channel population (Alzheimer et al., 1993; Brown et al., 1994; Mantegazza et al., 1998). The kinetics of the transient sodium conductance were adjusted to approximate the abrupt onset and height of the action potential (AP) in the IB neuron during simulated 150 pA current injection, by allowing for a faster and sharper activation and reducing the speed of inactivation: The activation kinetics were shifted 3.5 mV negative, the activation time constant reduced by 50% and the steepness of the steadystate activation function increased by reducing steepness factor *k* from 10 to 8 mV. The steady-state inactivation function was shifted 2 mV positive and the steepness increased by reducing the steepness factor *k* from 10.7 to 7 mV, while the inactivation time constant was increased by a factor 5. The reversal potential for sodium conductances was 0.05 V in the model.



Fig. 3.5: The functions describing the steady-state (in)activation and time constants of the model sodium conductances g_{NaP} and g_{NaF} . Fully drawn lines show the functions used in the model, whereas dashed lines describe the original functions from Traub et. al (2003) in cases where the functions were adjusted during the fitting of model response.

3.2.3.2 Potassium conductances

Initial attempts to implement the fast, slowly and very slowly inactivating potassium conductances described by Korngreen & Sakmann (2000) in the model resulted in either abolishment of the slow afterpotential following the initial burst, or a state of regular action potential firing. Instead the fast inactivating g_{KA} , slow inactivating g_{K2} and non-inactivating g_{KM} potassium conductances from Traub et al. (2003) were used, along with delayed rectifier potassium conductance and calcium-dependent conductances from the same publication. The potassium conductance reversal potential in the model was -0.09 V.

The time constant of the delayed rectifier potassium conductance g_{KDr} was increased by a factor 3, thereby prolonging the time until development of maximal current as well as the duration of the inward current. Thereby the width of the individual action potentials of the model neuron could be fit to the experimental data, along with the interspike interval. The time constant of the muscarinic potassium conductance g_{KM} was reduced by 25% to allow for a faster conductance de-activation following the burst fired during the simulated 150 pA current injection, thereby fitting the time course of the slow afterpotential.



Fig. 3.6: The functions describing the steady-state (in)activation and time constants of the model potassium conductances. Fully drawn lines show the functions used in the model, whereas dashed lines describe the original functions from Traub et. al (2003) in cases where the functions were adjusted during the fitting of model response.

In addition to the BK-type calcium (and voltage) dependent potassium conductance g_{KC} , a slower purely calcium dependent conductance g_{KAHP} was included to model a longer lasting inward current due to increased intra-cellular calcium-ion concentration following action firing. This current additionally accounted for the membrane potential





Fig. 3.7: The functions describing the steady-state activation and time constants of the model calciumdependent potassium conductances. The BK-type g_{KC} conductance kinetics are both voltage and calcium concentration dependent, with the effect of the calcium concentration dependent activation modelled as instantaneous. $[Ca^{2+}]_i$ is given in the so-called arbitrary units (Traub et al., 2003).

To provide necessary activation for the calcium-dependent n-gate of the g_{KC} and g_{KAHP} potassium conductances, the intracellular calcium concentration after inward currents through the modelled calcium channels was modelled 2 nm thick shell under the neuronal membrane using the following equation

$$\frac{d\left[Ca^{2+}\right]_{i}}{dt} = BI_{Ca} - \frac{\left[Ca^{2+}\right]_{i}}{\tau}$$

where the first term on right side describes the increase in concentration due to the total transmembrane calcium current in the compartment $I_{Ca.}$ The second term models the decay of the calcium concentration with the time-constant τ (100 ms in the somatic compartment, 20 ms in all dendritic compartments) due to different cellular processes like buffering and removal of calcium-ions. In the original publication the concentration was specified in "arbitrary units" (Traub et al., 2003) impeding a conversion to the GENESIS use of the SI-system. Instead the parameter *B* was fitted a value of 5.2•10⁴ to ensure a intracellular calcium concentration sufficient large to permit for activation of the [Ca²⁺]_i-dependent potassium conductances.

3.2.3.3 Calcium conductances

Only one type of high voltage activated (HVA) calcium conductance, a non-inactivating conductance g_{CaL} , was included in the model, as the different HVA calcium channel subtypes have been shown to possess very similar biophysical properties (Lorenzon & Foehring, 1995). The steady-state inactivation function was of the low voltage activated (LVA) calcium conductance g_{CaT} was shifted 5 mV negative to prevent a steady inward current at resting membrane potential, as a hyperpolarization of the neuron is generally necessary to release the LVA calcium currents from steady-state inactivation (Huguenard, 1996). The calcium conductance reversal potential in the model was 0.125 V.



Fig. 3.8: The functions describing the steady-state (in)activation and time constants of the model calcium conductances. Fully drawn lines show the functions used in the model, whereas dashed lines describe the original functions from Traub et. al (2003) in cases where the functions were adjusted during the fitting of model response.

3.2.3.4 Mixed cation conductance

The mixed cation hyperpolarization activated conductance g_H was implemented based on data from Williams & Stuart (2000). Steady state activation was shifted 6 mV positive to match activation of g_H during hyperpolarizing current pulses seen in the experimental data. The originally discontinuous equations governing the activation time constant were shifted 11 mV positive and joined at -71 mV. Additionally the time constant above -71 mV was increased by a factor 5 to match the time course of the membrane potential overshooting the resting value after offset of the hyperpolarizing current injections. The density of g_H was calculated individually for each individual dendritic compartment, resulting in a low density proximally and a higher density in the distal dendrites (Stuart & Spruston, 1998; Table 3.5), effectively resulting in a distribution that can be visualized as the mirror-reverse of the distribution of the specific membrane resistance shown in figure 4.3A. The reversal potential of the mixed cation conductance was -0.043 V.



Fig. 3.9: The functions describing the steady-state activation and time constant of the model g_H conductance. Fully drawn lines show the functions used in the model, whereas dashed lines describe the original functions from Williams & Stuart (2000).

The adjusted expressions for activation and inactivation kinetics of all voltage-gated conductances, as well as their fitted conductance densities in the different subdomains of the reconstructed neuronal morphology of the layer V IB pyramidal neuron are summarized in tables 3.3-5. All voltage-gated channel were implemented as *tabchannels* and simulations were done in hines-solver mode using the Crank-Nicholson implicit method of numerical integration with a simulation time-step of 50 µsec.

	Gates	Steady-state activation	Time constant (sec)
g _H	M = 1	$1 / (1 + \exp[(-0.085 - V) / -0.0061])$	$0.0011 \cdot (\exp[25 \cdot V + 6.68]) [V \le -0.071]$
Ŭ			$0.0059 \cdot (\exp[-27 \cdot V + 1.69]) [V \ge -0.071]$
g _{NaF}	m = 3	$1 / (1 + \exp[(-0.038 - V) / 0.008]$	$0.0125e-3 + (0.007e-3 \cdot exp[(0.03 + V) / 0.008]) [V \le -0.032]$
			$0.01e-3 + (0.0725e-3 \cdot \exp[(-0.03 - V) / 0.008]) [V \ge -0.032]$
	h = 1	$1 / (1 + \exp[(0.0574 + V) / 0.007]$	0.75e-3 + (5.75e-3 / (1 + exp[(0.0335 + V) / 0.01]))
g _{NaP}	M = 1	$1 / (1 + \exp[(-0.035 - V) / 0.01])$	$0.025e-3 + (0.014e-3 \cdot \exp[(0.027 + V) / 0.01])$ [V ≤ -0.027]
			$0.02e-3 + (0.145e-3 \cdot exp[(-0.027 - V) / 0.01]) [V \ge -0.027]$
g _{KDr}	M = 4	$1 / (1 + \exp[(-0.0295 - V) / 0.01]$	$0.75e-3 + (13.05e-3 \cdot \exp[(0.01 + V) / 0.01]) [V \le -0.01]$
			$0.75e-3 + (13.05e-3 \cdot exp[(-0.01 - V) / 0.01]) [V \ge -0.01]$
g _{KA}	m = 4	$1 / (1 + \exp[(-0.06 - V) / 0.0085])$	0.185e-3 + (0.5e-3 / ((exp[(0.0358 + V) / 0.0197]) + (exp[(-0.0797 - V) / 0.0127]))
			$0.5e-3 / ((exp[(0.046 + V) / 0.005]) + (exp[(-0.238 - V) / 0.0375])) [V \le -0.063]$
	h = 1	$1 / (1 + \exp[(0.078 + V) / 0.006]$	9.5e-3 $[V \ge -0.063]$
g _{K2}	m = 1	$1 / (1 + \exp[(-0.01 - V) / 0.017])$	4.95e-3 + (0.5e-3 / ((exp[(0.081 + V) / 0.0256]) + (exp[(-0.132 - V) / 0.018])))
-	h = 1	$1 / (1 + \exp[(0.058 + V) / 0.0106]$	60e-3 + (0.5e-3 / ((exp[(0.00133 + V) / 0.2]) + (exp[(-0.13 - V) / 0.0071])))
g _{CaT}	m = 2	$1 / (1 + \exp[(-0.056 - V) / 0.0062]$	0.204e-3 + (0.333e-3 / ((exp[(0.0158 + V) / 0.0182]) + (exp[(-0.131 - V) / 0.0167]))
-	h = 1	$1 / (1 + \exp[(0.085 + V) / 0.004]]$	$0.333e-3 \cdot \exp[(0.466 + V) / 0.0666] [V \le -0.081]$
			$9.32e-3 + (0.333e-3 \cdot exp[(-0.021 - V) / 0.0105])$ [V ≥ -0.081]

Table 3.3: Adjusted kinetic descriptions of active conductances using expressions for steady-state (in)activation and time constants as functions of membrane potential in Volts. [g_{NaF} = transient inactivating sodium conductance, g_{NaP} = persistent sodium conductance, g_h = hyperpolarization-activated mixed cation conductance, g_{CaT} = low voltage calcium conductance, g_{KDr} = delayed rectifier potassium conductance, transient inactivating potassium conductance g_{KA} , slowly activating and inactivating potassium conductance g_{K2}]

	Gates	Forward rate function (α)		
gкc	m = 1	$53 \cdot (\exp[(0.05 + V) / 0.011]) - \exp[(0.0535 + V) / 0.027]) [V \le -0.01]$		
		2000 • exp[(-0.0535 - V) / 0.027] [V \ge -0.01]		
	n = 1	min(0.004•[Ca ²⁺],1.0)		
g _{км}	m = 1	26.67 / (1 + exp[(-0.02 - V) / 0.005])		
g _{канр}	n = 1	$\min(0.1 \cdot [Ca^{2+}]_{i}, 10.0)$		
g CaL	m = 1	$1600 / (1 + \exp[-72 \cdot (V - 0.005)])$		
		Backward rate function (β)		
g _{кс}	m = 1	$2000 \cdot \exp[(-0.0535 - V) / 0.027] - \alpha [V \le -0.01]$		
	n = 1	$0 [V \ge -0.01]$		
g _{км}	m = 1	13.33 / (1 + exp[(-0.043 - V) / 0.018])		
g _{канр}	n = 1	10.0		
g _{CaL}	m = 1	$(20e3 \cdot (0.0089 + V)) / (exp[(0.0089 + V) / 0.005] - 1)$		

Table 3.4: Adjusted kinetic descriptions of active conductances using expressions for forward and backward rate functions as functions of membrane potential in Volts. $[g_{CaL} = \text{high voltage calcium conductance}, g_{KM} = \text{muscarinic}$ receptor-supressed potassium conductance, $g_{KC} = \text{fast voltage and } [Ca^{2+}]_i$ -dependent BK-type potassium conductance, and a slow $[Ca^{2+}]_i$ -dependent potassium conductance responsible for afterhyperpolarization g_{KAHP}]

	Soma	Basal dendrites	Apical shaft	Proximal apical dendrites	Medial apical dendrites	Distal apical dendrites
gн	$g_h = g_{h,som}$	$a + \frac{g_{h,distal} - g}{1 + e^{(500\mu m - distance)}}$	$\frac{h,soma}{st}$ (g _{h,som}	$m_{a} = 0.15, g_{h,distal} = 6.0;$	<i>dist</i> = distance to so	ma)
g _{NaF}	4400	350	350	350	350	62.5
g _{NaP}	0.0032* g _{NaF}	0.0032* g _{NaF}	0.0032* g _{NaF}	0.0032* g _{NaF}	0.0032* g _{NaF}	0.0032* g _{NaF}
g _{KDr}	1250	350	350	350	350	0
gka	300	20	300	20	20	20
g _{K2}	1	1	1	1	1	1
g CaT	1	1	1	1	1	1
g _{KC}	22.5	22.5	22.5	22.5	22.5	0
g _{км}	29	29	29	29	25	9.25
g KAHP	1	1	1	1	1	1
σαι	5	3	3	3	3	15

Table 3.5: Fitted densitities of active conductances (Sm^{-2}) . The density of g_h was calculated for each individual compartment using the given equation.

3.2.4 Model behavior

3.2.4.1 Comparison to the fitted experimental I-V responses

As can be seen in figure 3.10, the model fits the experimentally recorded responses to current injection well with some smaller deviations. The largest discrepancy is a gradually increasing steepness of depolarization before the action potential onset, which fails to capture the sharpness of the voltage inflection seen in the experimentally recorded traces. This can be attributed to the simulation of action potential initiation in the large single compartment soma with a slow charging of a relatively large membrane capacitance as opposed to simulations including a reconstructed axon hillock and initial segment as the site of action potential initiation (Mainen et al., 1995). Smaller deviations are to be expected when fitting to this type of data, since the voltage traces from the experimental I-V characterizations stem from single trial experiments. It would be desirable to fit the model responses to a set of repeated characterizations, which would provide a set of statistical boundaries for natural trial-to-trial variability of the responses.



Fig. 3.10: Comparison of the simulated (-) and experimentally recorded (--) responses to 17 somatic current injections ranging from -700 pA to 150 pA in steps of 50 pA with stimulus onset at t = 0.05 sec and offset at t = 0.250 sec.

3.2.4.2 Responses to non-fitted conditions

To further test the model, the responses were tested under two nonfitted conditions. Mantegazza et al. (1998) report an increase in bursting tendencies of IB neurons from rat sensorimotor cortex after treatment with anemone toxin (ATX-II), a selective inhibitor of sodium current inactivation, which leads to an increase of the persistent fraction of the sodium current. The model response to a 200 ms 150 pA depolarizing current injection after a 25% increase of g_{NaP} conductance density shows one additional action potential in the initial burst, consistent with experimental data (Mantegazza et al., 1998; Figure 3.11A).



Fig. 3.11: A. Response to 150 pA current injection shows one additional action potential in the initial burst when g_{NaP} is increased by 25%. B. Repeated burst firing during simulated 300 pA somatic current injection.

Additionally, when increasing the amplitude of the simulated current injection from the action potential eliciting 150 pA to 300 pA, the model shows repetitive burst firing as reported for layer V IB pyramidal neurons from rat somatosensory cortex (Zhu & Connors, 1999; Figure 3.11B).

3.2.4.3 Action potential back-propagation and the role of the apical tuft

The back-propagation of the axon-initiated action potential into both basal and apical dendrites of layer V pyramidal neurons has been investigated in several experimental and modelling studies (Antic, 2003; Berger & Lüscher, 2003; Larkum et al., 1999; Larkum et al., 2001; Rapp et al, 1996), and is likely involved in coding and processing of information on the single neuron level, allowing for association of inputs in different parts of the dendritic tree on a millisecond scale. Stuart et al. (1997) characterized the process of backpropagation in detail and reported lasting dendritic depolarizations in the distal apical dendrites following burst firing in layer V pyramidal neurons. When visualizing the membrane potential in the entire constructed pyramidal neuron model at different instants following burst firing, the time course of backpropagation can easily be seen (Figure 3.12).



Figure 3.12: Top: Enlarged view of the time course of the somatic action potential under control conditions and with reduced g_{CaL} in the apical tuft. A-H: Visualization of the membrane potential in the entire model neuron at 8 instants during the burst firing response to a simulated 150 pA somatic current injection. Corresponding times are marked on the top graph.

After initiation in the soma (Figure 3.12B), the first action potential back-propagates into the dendritic tree where the peak potential is reached after ~1 ms at distances of ~600 μ m along the apical dendrite as reported from the experiments (Stuart et al., 1997). Following the second action potential in the burst (Figure 3.12E), a lasting plateau potential is seen in the apical tuft (Figure 3.12E-H), whereas the medial apical dendrites slowly repolarize, first reaching the somatic potential after ~6 ms (Figure 3.12F) and even slightly undershooting it (Figure 3.12G-H) consistent with the experimental reports. Decreasing the distal conductance density of the non-inactivating HVA calcium conductance g_{CaL} by a factor of 15 abolished the lasting potential, as seen in the simulated somatic potential traces (Figure 3.12 top), complying with data from experiments involving pharmacological blocking of calcium conductances (Larkum et al., 1999; Larkum et al., 2001; Stuart et al., 1997).

3.3 Summary

The model reproduces the behavior of the fitted layer V pyramidal neuron responses with a very good approximation in spite of the very limited amount of detailed kinetic data on voltage-gated conductances from layer V IB neurons from rat somatosensory cortex, which necessitated the adaptation of conductance equations from other modelling studies. Realistic responses to the two tested non-fitted conditions (Figure 3.11), and in particular the precise reproduction of the complex back-propagation behavior of the action potential into the apical dendrites without additional tuning of the model (Figure 3.12) support the conclusion that the neuron model will be useful for further investigations of local circuit connectivity in the rat barrel cortex.

4 Analysis of direct glutamate induced activation of neurons in slice

In the following 2 chapters results from using the implemented detailed layer V IB pyramidal neuron model in conjunction with experimental data to investigate connectivity in local microcircuits of the rat barrel cortex will be presented. The experiments were performed collaborators at the Institute of Physiology and C. & O. Vogt Brain Research Institute, Heinrich Heine Universität Düsseldorf, and parts thereof have previously been published (Schubert et al., 2001; Schubert, 2003).

4.1 Recordings of directly induced glutamate activation in layer 5

pyramidal neurons

The effects of direct stimulation with the excitatory neurotransmitter l-glutamate on layer V pyramidal cells were investigated experimentally (Schubert et. al, 2001; Schubert, 2003) using an experimental set-up combining whole-cell patch clamp recording, infrared video microscopy and UV flash induced release of the excitatory neurotransmitter glutamate from an inert *caged* form (Kötter et. al, 1998; Schubert et al., 2001).

300 µm thick coronal slices of cortex from male Wistar rats (postnatal days 18-22) containing barrel cortex were placed in a recording chamber containing modified *artificial cerebro spinal fluid* with a lowered concentration of calcium-ions and increased concentration of magnesium-ions (low Ca^{2+/}high Mg²⁺ ACSF). The recording chamber was placed under an upright microscope that rested on a mobile stage. Individual layer V pyramidal neurons in a barrel associated column were identified using infrared illumination from below the slice, projected through the microscope optics to an infrared sensitive CCD camera and visualized on a monitor.

After establishing a stable whole cell patch recording and characterizing the firing properties of the recorded neuron under current clamp conditions (see previous chapter), neuronal responses to the release of caged glutamate (added to the chamber solution) by UV flash induced uncaging were recorded (Figure 4.1).



Fig. 4.1: Simplified illustration of the experimental set-up. A single visually identified neuron is recorded in whole cell patch clamp configuration. UV flashes are focused onto the slice through the microscope optics to release glutamate from the ester-bound caging group (modified from Kötter et al., 1998).

Flashes from a pulsed xenon arc lamp with a duration of ~500 µsec were directed by a fiber optics cable through a short pass filter mounted on the epifluorescence port of the microscope and focused on 50µm x 50µm large areas on the submerged cortical slice. The resulting UV flash cleaves the ester bond between the carboxy-nitrobenzyl (CNB) caging group and glutamate in the inert caged compound *L-glutamic acid*, γ -[α -carboxy-2-nitrobenzyl]-ester in a photolytic reaction, releasing free glutamate in the slice.



Fig. 4.2: Molecular structure of L-glutamic acid, γ -[α -carboxy-2-nitrobenzyl]-ester. The ester bond is indicated by the dashed line, with the carboxy-nitrobenzyl caging group at the bottom and the glutamate at the top(modified from Kötter et al., 1998).

By moving the computer controlled motorized stage of the microscope, glutamate could be released systematically in well-defined areas forming a stimulus grid, covering most of the dendritic tree of the identified neuron (Figure 4.3). As the recordings were carried out in low Ca^{2+/}high Mg²⁺ ACSF, synaptic transmission of action potentials from presynaptic neurons was blocked, ensuring that only direct activation of the glutamate receptors by the uncaged glutamate would induce responses in the recorded neuron.



А

Fig. 4.3: Stimulation grids overlaying reconstructed neuronal morphologies. A. A layer V IB pyramidal neuron overlaid by a grid of 10x25 release sites. B. A layer V RS pyramidal neuron overlaid by a grid of 10x20 release sites.

After recording the responses to flash stimulation of the entire grid (Figure 4.3), recorded neurons were filled with biocytin and the slices processed for reconstruction of the neuronal morphologies in the computer program NeuroLucida.



Fig. 4.4: Colorcoded maps of somatically recorded voltage responses in low $Ca^{2+}/high Mg^{2+} ACSF$, where color signifies the amplitude of the somatically recorded response after releasing glutamate in the given square. Recordings were made from the layer V IB (A) and RS (B) pyramidal neurons shown in figure 4.3. Action potential amplitude was truncated at 13 mV. Positions where AP firing was induced are indicated with an asterisk (*).

Figure 4.4 shows examples of response amplitude maps constructed from recordings from a layer V IB neuron (A) and a layer V RS neuron (B), and illustrates the general findings well: Somatic depolarizations were only recorded when stimulating fields of the stimulation grid covering dendrites of the recorded neuron, however not all fields containing dendrites yielded a somatic response. The restriction of responses to fields containing dendritic segments attest to the restriction of glutamate release to the focus area.

Action potentials could only be evoked perisomatic stimulation sites (close to the soma), and the response amplitude mostly decreased drastically with $\sim 100 \,\mu\text{m}$ distance from the soma. The strongest responses were generally recorded from areas containing proximal dendritic branches, whereas weaker responses were recorded after stimulation of distal dendrites. However, as can be seen in figure 4.4, there was no strict relationship between distance from soma and response amplitude.

4.2 Analysis of response amplitude dependence on dendritic depth in slice

Since the UV flash was focused on the depth of the recorded neuron soma through the microscope optics, the light intensity at this depth must be maximal leading to the highest yield of uncaged glutamate from the photolytic reaction. This would indicate a possible correlation between depth of the stimulated dendrites (relative to the focal plane) and the recorded response amplitudes.

To investigate this, the distribution of dendrites along with the soma position relative to the slice surface were calculated for the two reconstructed morphologies shown in figure 4.3, by correcting for the shrinkage incurred during the histological processing, measured as the thickness of the processed slice relative to the nominal thickness of 300 μ m cut for the experiments (Schubert et al., 2001).

	Nominal slice thickness	Thickness of processed slice	Shrinkage factor	Corrected soma depth
IB neuron	300 µm	220 µm	1.36	-24.5 μm
RS neuron	300 µm	236 µm	1.27	-46.5 µm

Table 4.1: Shrinkage factor and corrected soma depth obtained for the two reconstructed neurons.

Maps showing the depth of the most superficial dendrite for each site in the stimulation grid were constructed using the coordinates of the shrinkage corrected reconstructed morphologies (Figure 4.5 A-B) along with distributions of response amplitudes as a functional of superficial dendrite depth (Figure 4.5 C-D).



Fig. 4.5: The dependence of response amplitude on the depth of the most superficial dendrites in the slice. Colorcoded maps (A and B) show the depth of the most superficial dendrite at each stimulation square of the IB (A) and RS (B) layer V pyramidal neurons. The response amplitude elicited by flash induced release of glutamate in a square quickly diminished below the depth of the focal plane (C and D).

A steep drop in response amplitude following flash stimulation is seen for both the analyzed neurons when the targeted dendrites are positioned deeper in the slice than the

focal plane at the soma depth (Figure 4.5 C and D). Additionally the maps of superficial dendrite depth show that neighboring stimulus sites can contain dendrites at depths differing by up to 100 μ m, providing a possible explanation for the differences in recorded response amplitudes between neighboring stimulus sites shown in the amplitude maps (Figure 4.4). These features are valid for both neurons analyzed, but most prominent for the reconstructed layer V IB neuron, which also shows the largest difference in response failures (i.e. stimulated sites containing dendrites but showing no response) between sites above and below the depth of the focal plane.

4.3 Modelling direct glutamate induced responses in a layer V IB neuron

To test the apparent correlation between dendritic depth in the cortical slice and the elicited response amplitude, a model of the described flash experiment was constructed using the detailed layer V IB neuron model described in the previous chapter. Each compartment of the model was equipped with a model glutamate activated synaptic conductance receiving simulated flash input as the simulated slice was scanned in a 10x25 grid identical to experimental situation (Figure 4.3A).

Using the distribution of response amplitudes as a function of dendritic depth (Figure 4.5), a function scaling the activation strength for a given simulated synapse was constructed, using the upper boundary of the experimentally recorded response amplitudes while excluding the truncated action potentials (Figure 4.6). Since the model morphology was shrinkage corrected in the z-direction (depth coordinate) using only a factor of 1.15 to avoid artificial elongation of dendritic segments (see section 3.2.1), this scaling function was described in the coordinate space of the model morphology.



Fig 4.6: A. The response amplitude distribution in depth coordinates shrinkage corrected according to the single neuron model. Dashed line shows the amplitude limit used for the model scaling function. B. The normalized synaptic conductance activation scaling function based on the amplitude distribution excluding the truncated action potential amplitudes.

The release of glutamate following the UV flash stimulation has been reported to occur with almost immediately with a time constant of ~21 µsec (Cheng et. al, 2002; Wieboldt et al., 1994) but little is known about the development of the concentration profile after release. To approximate the experimentally recorded responses to the release of glutamate, the time constants of the model synaptic conductance were set to $\tau_1 = 0.1$ ms and $\tau_2 = 100$ ms with a simulated activation by a square pulse with a duration of 5 ms. The time constants of the synaptic conductance are not taken to reflect any physiological properties of the actual channel, but model the effect of the flash released glutamate in combination with the square pulse stimulus.



Fig. 4.7: Example of perisomatically located flash induced responses from simulation and experiment.

The length of the individual compartments in the model neuron is not constant but determined by the criterion requiring each compartment to be maximally $0.01-0.05\lambda$ to ensure isopotentiality during the simulation. This means that the parts of the morphology

with the smallest diameter dendritic branches (e.g. the apical tuft) contain a larger number of compartments than parts with relatively large diameter branches like the proximal apical dendrite (see section 3.1). Consequently the probability p_{act} of activating the model synaptic conductance in a given compartment was made proportional to the compartment length divided by 10 µm. This length was selected, as it was longer than the maximal compartment length.

$$p_{act} = p_{base} \frac{compartment _length}{10 \, \mu m}$$

The base probability p_{base} was set to 1. This scaling of the activation probability avoided an artificial increase in the amount of stimulation to small diameter dendritic branches.

Each model synaptic conductance was initially given a maximal conductance $g_{max,syn} = 25$ nS and a reversal potential $E_{syn} = 0.0$ V. Activation was determined randomly for all dendritic compartments contained in a stimulation square using the probability described above, and the maximal activation was scaled according the depth in the slice for each individual synaptic conductance using the function given in Figure 4.6 B.

4.4 Simulation results

Results from initial simulations proved it necessary to increase the maximal conductance of model synapse conductances located on apical dendrites beyond the main apical dendritic shaft in order to induce measurable somatic responses. This was accomplished using a gradual increase of 5 nS/100 μ m for model synaptic conductances located in apical dendritic compartments more than 75 μ m away from the soma.

Figure 4.8 shows the results obtained from three simulations of the flash experiment different initialization seeds for the random number generator determining the activation of the individual model synapse conductances. As can be seen by comparing the simulated maps with the original experimentally obtained amplitude map from the layer V IB neuron in Figure 4.4A, activation of model synaptic conductances as a function of their depth in the slice produces maps that preserve the features reported from the experiments: Activations leading to action potential firing are located close to the neuron soma, and the response amplitude decreases rapidly outside of the most perisomatically located stimulus sites, with individual larger responses at sites containing basal and apical oblique dendrites.

Additionally the more patchy activation patterns found in the medial and distal apical dendrite domains is also reproduced by the simulation.



Fig. 4.8: Amplitude maps and response amplitude distributions as a function of superficial dendrite depth for simulations with three different random seeds. The amplitude distributions are mapped to the coordinates of the original shrinkage corrected slice measurements, with simulated distribution (o) overlaid on the experimental distribution (+). A minimal response threshold was set at 0.2 mV according to the smallest measurable experimental response amplitudes.

The response amplitude distributions from the simulations (Figure 4.8A-C) follow the general shape of the experimental amplitude distribution (Figure 4.5C), but reveals that the model fails to produce responses for stimulation sites only containing dendrites at depths

below ~100 μm close with amplitudes close to the set threshold for minimal responses of 0.2 mV.

The model does not capture the exact shape of the experimentally obtained map or the exact position and amplitude of individual recorded responses. It is possible that this could stem from discrepancies between dendritic positions in the experiment and the model due to either differential shrinkage during the histological procedures or minor inaccuracies in the alignment of the reconstructed morphology used in the simulations (Jaeger, 2001).

4.4.1 Effects of (x,y) coordinate variations

To investigate the effect of minor variations of the dendritic positions on the amplitude maps, a series of simulations systematically were performed varying the x- and y- coordinates of the stimulation grid alone or simultaneously. All variations were done with the same random seed used for the amplitude map in Figure 4.8A.

All coordinate variations resulted in minor differences in the shape of the map, particularly in areas only containing smaller amount of superficial dendrites such as the more distal apical oblique dendrites and the apical tuft (Figure 4.9). In addition, stimulation sites producing larger response amplitudes (> 4 mV, including action potential firing) varied both in numbers and positions as dendritic branches were associated with different areas of the stimulation grid.



Fig. 4.9: Systematically varying stimulation grid position relative to the neuronal morphology by $\pm 10\mu m$ in the x and y directions affect the positions and amplitude of individual responses to simulated flash stimulation.

Neither of these variations had any larger effect on the response amplitude distributions as a function of superficial dendrite depth, which were all within the same boundaries as those described for the three differently seeded simulations in Figure 4.8.

4.4.2 Effects of varying focal depth

Due to the apparent strong dependence of dendritic on position close to the focal plane of the glutamate releasing flash (Figure 4.5 C-D), the effect of minor variations of focal depth in the simulation of the flash experiment was investigated. Setting the focal depth 5 μ m deeper in the model (a change of -5μ m) increased the number of stimulation sites inducing action potential firing in the simulation from two to four (compare 4.10A to 4.8A).



Fig. 4.10: Increasing the depth of the focal plane by 5 μ m leads to increased action potential firing in the model (A-B), while moving the focal plane 5 μ m closer to the surface of the simulated slice abolishes action potential firing (C-D). Response amplitude distributions (o) are mapped to the coordinates of the original shrinkage corrected slice measurements and overlaid with the experimental distributions (+).

Moving the focal plane 5 μ m up (more superficial in the simulated slice experiment) abolishes action potential firing in the model. In both cases the distribution of response amplitudes at larger dendritic depths remained unchanged by the change in focus depth.

4.5 Summary

Simulations of somatically recorded neuronal responses to direct stimulation of synaptic conductances following UV flash induced release of caged glutamate produce realistic response amplitude maps, when the strength of activation is made strongly dependent on the distance of the activated neuronal compartment from the focal plane of the stimulation. The model distribution of response amplitudes as a function of the depth of most superficial dendrite in a stimulated square show a good correspondence to the experimental distributions, but fails to produce near threshold responses at depths exceeding 100 µm.

Additional simulations showed the sensitivity of position and amplitude of individual induced responses to variations in the relative position of the reconstructed morphology and the stimulation grid. The possible distortions of reconstructed morphologies caused by differential shrinkage during morphological processing (Jaeger, 2001), offers an explanation of the inability of the model to capture the exact locations and amplitudes of individual responses in the experimentally obtained maps. It is however likely that these distortions are largest in the along the z-axis (depth of the slice) where differential shrinkage during post experimental tissue processing, as well compression by the slicing methods used to produce the slices, can significantly distort the shape of the slice (Gardella et al., 2003). Since the responses obtained in simulations using the reconstructed morphology succeeded in producing realistic maps and amplitude distributions, these effects are unlikely to be large in our case, but possibly large enough to influence the position and strength of individual responses, as well as the near threshold responses from dendrites deep in the slice.

The dependence of induced responses from model synaptic conductances close to the focal plane was found to be very strong when using the scaling function based on the experimental distribution (Figure 4.6), with an only $\pm 5 \,\mu\text{m}$ displacement (in model coordinate space) sufficient to double or abolish action potential firing in the simulation. This displacement corresponds to $\sim \pm 7 \,\mu\text{m}$ in the shrinkage corrected slice.

The crude approximation of the glutamate activation in the simulations by a combination of a short, square activation pulse and a long decay time of the model conductances (Figure 4.7) proved sufficient to produce realistic amplitude maps and distributions. It is however evident that the simulated response has a longer rise time than the experimental trace whereas the decay phases are very similar. This could suggest that the actual glutamate

profile has an initial larger peak than the simulated square pulse, followed by a longer lasting decaying phase providing lasting activation of the glutamate receptors.

In order to evoke realistic responses to simulated glutamate release from sites on the apical dendrites more distal to the soma than ~75 μ m, a gradual increase in maximal synaptic conductance had to be incorporated into the model. This is in agreement with reports of increased densities of the AMPA (α -amino-3-hydroxy-5-methyl-4-isooxalone propionic acid) glutamate receptor subtype along the apical dendrite of CA1 pyramidal neurons from rat hippocampus and layer V pyramidal neurons from the rat neocortex (Andrásfalvy & Magee, 2001; Dodt et al., 1998). Since the direct flash stimulation experiments were carried out in low Ca^{2+/}high Mg²⁺ ACSF, contributions from activation of the NMDA (N-methyl-D-aspartate) glutamate receptor subtype are likely negligible, due to a reduced driving force in the lower calcium-ion environment along with an strong enhancement of the voltage-dependent magnesium-ion block of this channel type (Hille, 1992).

5 Investigating the local connectivity of layer V IB pyramidal neurons

In a series of experiments, using the experimental setup described in the previous chapter, the positions of neurons forming functional connections with layer V IB neurons were mapped. Briefly, while recording from a single identified layer V IB neuron in a whole cell patch clamp configuration, caged glutamate was released by UV flash induced photolysis in a grid of 50x50 µm stimulation sites covering at least two adjacent columns in a coronal slice of the rat barrel cortex (Schubert et al, 2001). These recordings were performed in standard ACSF (as opposed to the experiments investigating direct inputs in low Ca²⁺/high Mg²⁺ ACSF), glutamate induced depolarizations sufficiently large to induce action potential firing in neurons forming synaptic connections with the recorded neuron would results in post synaptic potentials (PSPs) being recorded. By systematically scanning the entire stimulation grid (Figure 5.1), an extensive mapping of the strength and extent of connections originating from neurons in different layers and columns could be carried out.



Figure 5.1: Schematic diagram of a 15x30 grid of 50x50µm stimulation sited covering two adjacent columns in the cortical slice including the identified layer V IB pyramidal neuron recorded from. Due to the curvature of the cortex, the position of the intralaminar borders are different in the grid covering the adjacent barrel-related column.
Stimulation of sites containing dendritic segments of the recorded neuron additionally resulted in direct flash induced activation (see previous chapter), however these could be separated from the synaptic input based on differences in the delay-to-onset of the induced responses (Schubert et al., 2001). Layer V IB pyramidal neurons were found to receive excitatory inputs from all layers in both their column of origin and the neighbouring column, with the largest and most continuous number of EPSP (excitatory post synaptic potential) inducing stimulus locations in the infragranular layers and more patchy input originating from the granular and supragranular layers. A very limited number of inhibitory inputs (from < 2% of all stimulated sites) could primarily be induced from stimulus locations close to the recorded neuron in layers II/III, IV and V and will not be treated further here. Excitatory inputs strong enough to induce AP firing in the recorded neuron from a slightly (~ 1-2 mV) holding potential originated from stimulation sites restricted to intracolumnar layers IV and V, as well as transcolumnar layer IV.

To quantify the strength and extent of excitatory activity recorded after UV flash-induced release of caged glutamate at intra- and transcolumnar sites, the percentage of sites evoking EPSPs in the recorded neuron, as well as the mean and standard deviation of the evoked EPSPs amplitudes and integrals were calculated using the data from recordings of seven layer V IB pyramidal neurons. Responses with induced action potential firing had their amplitude truncated above 13 mV and were excluded from the EPSP integrals.

Intracolumnar stimulation	Activation (% of stimulated sites)		ar Activation EPSP Amplitude (% of stimulated sites) (mV)		EPSP integrals (mVs ⁻¹)	
Site	Mean	SD	Mean	SD	Mean	SD
II/III	24.4	19.0	0.86	0.59	0.0385	0.0377
IV	38.6	20.7	1.90	3.36	0.0596	0.1369
V	56.9	11.3	3.26	4.68	0.0403	0.0506
VI	89.0	9.5	1.45	1.50	0.0715	0.1012

Transcolumnar	Activation		EPSP Amplitude		EPSP integrals		
stimulation site	(% of stimu	lated sites)	(m	V)	(mVs ⁻¹)		
	Mean	SD	Mean	SD	Mean	SD	
II/III	16.8	10.2	0.60	0.35	0.0249	0.0234	
IV	29.5	9.9	2.14	3.42	0.0486	0.0797	
V	31.8	9.1	0.77	0.46	0.0275	0.0258	
VI	60.5	12.8	0.98	0.70	0.0437	0.0468	

Table 5.1: Quantification of EPSPs induced by stimulating intracolumnar sites

Table 5.2: Quantification of EPSPs induced by stimulating transcolumnar sites.

5.1 Detailed connectivity of layer V IB pyramidal neurons

As mentioned in chapter 1, detailed knowledge of the locations and characteristics of individual synapses on the dendrites of layer V IB pyramidal neurons in rat barrel cortex is very limited. Extensive investigations have only been carried out on neighbouring connected layer V IB cells and to a lesser extent for synapses established by layer II/III pyramidal neurons (Markram et al., 1997; Thomson & Bannister, 1998), since the identification and recording of connected neurons, followed by the reconstruction of morphologies and exact synaptic locations is both complicated and time-consuming.

Experiments using stimulations with current injection waveforms simulating the time course of EPSPs at different positions along the main apical dendrites of layer V pyramidal neurons have however established a stimulus-distance dependent increase in the 20-80% rise time (the time span of the increase from 20 to 80% of maximal amplitude) of somatically recorded depolarizations (Williams & Stuart, 2000).

Consequently, the 20-80% rise times for the first arriving and clearly distinguishable somatically recorded EPSPs were extracted for the different intra- and transcolumnar stimulation sites listed in tables 5.1 and 5.2. The resulting rise time distributions plotted as normalized histograms are shown in figure 5.2.



Fig. 5.2: Histograms showing the normalized distributions of somatically recorded EPSPs 20-80% rise times following flash-induced release of caged glutamate at stimulation sites located intra- and transcolumnarly in the different cortical layers. Bin size = 1 ms.

The rise time distributions (Figure 5.2) show a predominance of somatically recorded EPSPs with rise times shorter than 5 ms from stimulation sites in granular and infragranular layers (> 90%), with only intra- and transcolumnar stimulation in layer II/III

resulting in a larger fraction of responses with longer rise times (30% and 15% respectively).

Taking the EPSP with minimal rise time of 0.44 ms to originate from synapses located at the soma, and the EPSP with a maximal rise time of 14 ms to originate from synapses located at a distance of \sim 1200 µm from the soma in the distal apical tuft (Figure 5.1), results in a factor slope factor of

$(14ms - 0.44ms)/1200 \mu m \approx 90 \mu m / ms$

for calculating the theoretical distance to the position of a synapse from the soma, based on the 20-80% rise time of the recorded somatic EPSP. Applying this factor to the extracted rise times for all somatically recorded EPSPs results in the normalized synaptic distance distribution shown in figure 5.3.



Fig. 5.3: Normalized distributions of synaptic distances determined from the 20-80% rise times of somatically recorded EPSPs based on a theoretical increase in distance from the soma of 90 μ m/ms increase in rise time. Bin size = 50 μ m.

The calculated synaptic distance distributions indicate a predominant restriction of synaptic contacts formed on the layer V IB pyramidal neurons to basal, proximal apical and apical oblique dendrites within layer V (Fig. 5.1), with the exceptions being intra- and transcolumnar sites in layer II/III also forming synaptic contacts at distances corresponding to sites along the apical dendrites in layers IV and II/III. It furthermore appears that the positions of synapses on the layer V IB cells are similar for both intra- and transcolumnarly located sources in the same layer.

5.2 Modelling local synaptic inputs to layer V IB pyramidal neurons

In order to test the theoretically calculated distributions of synapses formed by local presynaptic neuronal populations on layer V IB pyramidal neurons in the rat barrel cortex, a model was constructed, based on the experimental situation described above.

The previously described detailed compartmental model of a layer V IB neuron recovered after recordings mapping direct and synaptic inputs after UV flash-induced release of caged glutamate (Schubert et al., 2001) was equipped with a single synapse in each neuronal compartment. To emulate the combined effect of activation of both the AMPAR (AMPA receptor) and NMDAR (NMDA receptor) subtypes of glutamate receptor activated channels, the synaptic conductances were implemented with an activation time constant $\tau_{act} = 0.2$ ms reported for AMPAR channels (Markram et al., 1997), whereas the decay time constant raised from 1.5 ms to $\tau_{decay} = 6.0$ ms mimicking the <20% reported contribution to the EPSP time-integral of NMDAR channels at potentials close to the resting membrane potential (Markram et al., 1997). The conductances were simulated with a peak conductance of $g_{max} = 225$ pS and a reversal potential $E_{Glu} = 0.0$ mV.

As most recordings only revealed a single EPSP in response to the release of caged glutamate, the simulated release of caged glutamate was assumed to activate only one presynaptic neuron. This assumption is furthermore supported by the correspondence of the average EPSP amplitude (excluding AP responses) of responses induced from stimulation sites in intracolumnar layer II/III and V (Table 5.1) to the experimentally determined ranges for single pairs of synaptically coupled neurons (Markram et al., 1997; Thomson & Bannister, 1998),

Synaptic connection	Amplitude from	Amplitude from mapping
	paired recordings	with caged glutamate
II/III→V	$0.8 \pm 0.6 \text{ mV}^1$	0.86±0.59 mV
$V \rightarrow V$	$1.3 \pm 1.1 \text{ mV}^2$	1.07±0.77 mV

Table 5.3: Comparison of EPSP amplitudes obtained from paired recording and mapping with release of caged glutamate. ¹Thomson & Bannister (1998). ²Markram et al. (1997).

Synaptic connections were established with the implemented synaptic conductances in different compartments using a probabilistic connection routine. For each intra- and transcolumnar layer, a set of five probabilities for five different dendritic domains were adjusted to achieve synaptic distance distribution profiles approximating the calculated distributions in figure 5.3, with an average number of close to 5 connections made per

presynaptic stimulation site, similar to the 5.5 ± 1.1 average reported for connections between pairs of identified layer V IB pyramidal neurons (Markram et al., 1997).

The normalized distributions of the geometric distance from the soma to individual compartments containing model synaptic conductances in five dendritic domains are shown in figure 5.4.



Fig. 5.4: Normalized distributions of geometric distances between the model neuron soma and individual compartments in the five separated dendritic domains. Bin size = $50 \mu m$.

In the simulations, established synaptic connections were activated by a single simulated action potential, and the g_{max} of each individual model conductance was scaled by a base weight plus a factor drawn randomly from a Gaussian distribution. The weight distributions were fitted separately for each intra- and transcolumnar stimulation site type to produce realistic EPSP responses in the model neuron.

5.4 Simulation results

Below the pooled results from five simulations each simulating the activation of sites in all intra- and transcolumnar cortical layers, in a manner similar to the systematic scanning of the stimulation grid used in the experiments, are presented. Each simulation was initialized with a different random seed. For each stimulation site in the simulated slice, the activation fractions given in tables 5.1 and 5.2 were used as a probability to determine whether a synaptic connections to the layer V pyramidal neuron should be established.

5.4.1 Synapse distributions

Initially, simulations including only the connectivity routines were performed to establish realistic distributions of synaptic connections to the layer V IB pyramidal neuron model. The distance distributions from the simulation generally approximate the calculated distributions well, however some systematic deviations are seen at distances of $300-500 \mu m$, particularly in the connections made by presynaptic populations in layer II/III (Figure 5.5). These deviations stem from the automatic division of the neuronal morphology into compartments based on the passive membrane properties of the neuron model (see section 3.2.1), resulting in larger compartments and thus fewer model synaptic targets in parts of the apical dendrite with large diameter.



Fig 5.5: Normalized population histograms comparing the modelled synaptic target distances with the distribution determined from the experimentally obtained 20-80% rise times. The results from the modelled synapse positions are pooled from all five simulations.

The mean and standard deviation for synapses made on each dendritic domain was subsequently calculated for each of the presynaptic populations, along with the mean and standard deviation of the total number of synapses for a given connection type (Tables 5.4 and 5.5).

Intracolumnar stimulation	Basal d	endrites	Proxima	al apical lrites	Obliqu deno	e apical lrites	Media deno	l apical lrites	Distal denc	apical lrites	Тс	otal
Site	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Layer II/III	0.77	0.81	0.38	0.55	2.03	1.34	1.23	1.21	0.37	0.63	4.79	2.13
Layer IV	1.71	1.07	0.17	0.38	2.54	1.92	0.17	0.38	0.0	0.0	4.60	2.02
Layer V	2.02	1.51	0.08	0.28	2.26	1.42	0.12	0.33	0.05	0.1	4.67	2.08
Layer VI	1.57	0.95	0.49	0.66	2.40	1.35	0.60	0.50	0.0	0.0	5.06	1.45

Table 5.4: Total numbers of synapses made from intracolumnar sources onto each of the five model dendritic domains.

Transcolumnar stimulation	Basal d	endrites	Proxima	al apical lrites	Obliqu deno	e apical lrites	Media deno	l apical Irites	Distal denc	apical Irites	Тс	otal
site	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Layer II/III	1.40	1.29	0.31	0.68	1.86	1.32	0.88	0.87	0.01	0.28	4.52	1.99
Layer IV	1.16	1.01	0.48	0.63	2.89	1.95	0.41	0.78	0.02	0.13	4.96	1.96
Layer V	1.95	1.41	0.07	0.30	2.36	1.41	0.21	0.41	0.0	0.0	4.59	1.94
Layer VI	2.06	1.36	0.0	0.0	2.06	1.64	0.69	0.47	0.0	0.0	4.81	227

Table 5.5: Total numbers of synapses made from transcolumnar sources onto each of the five model dendritic domains.

As can be seen from tables 5.4 and 5.5, the distribution of synapses reflect the preliminary interpretation of the calculated distances based on the rise times, with the largest number of synapses located on the basal and apical oblique dendrites of the layer V pyramidal neuron model. The variations in total number of synapses stem from the use of the probabilistic connection routine with very low (< 0.1%) probability for making connections to any given model synapse. This was necessary to ensure a reasonable variability of synapses being activated, and not simply the first synapses encountered in each of the five dendritic domains. Figure 5.6 illustrates the spatial distribution in the x-y plane of synapses contacted by all presynaptic stimulation sites in a single simulation.



Fig. 5.6: Scatter plots of all synaptic contacts made in the course of a single simulation from the different populations of presynaptic stimulation sites. A. Intracolumnar layer II/III. B. Intracolumnar layer IV. C. Intracolumnar layer V. D. Intracolumnar layer VI. E. Transcolumnar layer II/III. F. Transcolumnar layer IV. G. Transcolumnar layer V. H. Transcolumnar layer VI.

5.4.2 Simulated EPSP responses

After the synaptic distributions were established as described above, test simulations were run while fitting a distribution of synaptic weights for each presynaptic neuron population in order to approximate the experimentally recorded EPSP responses (Tables 5.6 and 5.7). The weight for an established connection was given as base weight plus a random component drawn from a Gaussian distribution with a fixed standard deviation and zero mean. Additionally a fixed upper boundary set the maximal deviation from the base weight.

Intracolumnar stimulation Site	Base weight	SD of Gaussian component	Maximal deviation
Layer II/III	0.45	0.30	2.0
Layer IV	0.6	0.5	17.0
Layer V	0.55	0.35	2.0
Layer VI	0.25	1.8	10.0

 Table 5.6: Synaptic weight distributions for intracolumnar stimulation sites.

Transcolumnar stimulation Site	Base weight	SD of Gaussian component	Maximal deviation
Layer II/III	0.35	0.1	1.5
Layer IV	0.9	0.6	17.0
Layer V	0.4	0.2	0.25
Layer VI	0.5	0.35	6.0

Table 5.7: Synaptic weight distributions for transcolumnar stimulation sites.

The fitted distributions of weights do not necessarily reflect the actual physiological variability between conductances of individual synaptic contacts, but should be seen as a scale for the impact of a synaptic connection including all contacts, given that the variability of the number synaptic contacts made in the model was not set to reflect an experimentally determined range.

The peak amplitudes and time integrals of the EPSP responses in the layer V pyramidal neuron model were extracted and pooled from the five differently seeded simulations run with fitted synaptic weight distributions.

Intracolumnar	EPSP an	nplitude	EPSP integral		
stimulation	(m	V)	(mVs^{-1})		
Site	Mean	SD	Mean	SD	
Layer II/III	0.88	0.49	0.027	0.015	
Layer IV	1.15	0.6	0.036	0.019	
Layer V	0.93	0.54	0.029	0.017	
Layer VI	1.52	1.2	0.047	0.037	

Table 5.8: Mean and standard deviations of thepeak amplitudes and integrals of the simulatedEPSP responses from intracolumnar stimulation.

Transcolumnar stimulation	EPSP ar (m	nplitude V)	EPSP integral (mVs ⁻¹)		
Site	Mean	SD	Mean	SD	
Layer II/III	0.54	0.26	0.017	0.008	
Layer IV	1.46	1.04	0.046	0.033	
Layer V	0.73	0.37	0.023	0.012	
Layer VI	0.92	0.54	0.028	0.017	

Table 5.9: Mean and standard deviations of thepeak amplitudes and integrals of the simulatedEPSP responses from transcolumnar stimulation.

A direct comparison of the calculated means and standard deviations of the EPSP integrals to the experimental values (Tables 5.1 and 5.2) showed that the integrals of the simulated responses were too small, deviating by up to 35% for the means and 75% for the standard deviations.

To compare the distributions of the simulated amplitudes to the experimental data, a twotailed Student's t-test was performed, comparing the experimentally obtained peak amplitudes for each intra- and transcolumnar population to the individual values extracted from the simulation data. The t-tests showed no significant difference between experiment and simulation for the peak amplitudes from stimulation in intracolumnar layer II/III (p =0.86) and VI (p = 0.67) and transcolumnar layers II/III (p = 0.26), V (p = 0.49) and VI (p =0.44). Significant differences were found between the peak amplitudes from experiment and simulation for stimulations in intracolumnar layers IV (p = 0.02) and V (p = 0.0001), as well as transcolumnar layer IV (p = 0.04).

The significant differences in peak amplitude distributions between experiment and model stem from the inability of the simulations to induce action potential firing in the layer V pyramidal when simulating the UV-flash induced release of caged glutamate in both intraand transcolumnar layer IV, as well as intracolumnar layer V. Attempts to fit the action potential inducing responses by further adjustment of the weight distributions for the mentioned connection types proved unsuccessful in the current model setup. Possible solutions to this would be further analysis of rise time distributions to establish whether stimulation sites at different distances induced different responses (and thus have different connectivity distributions) or changing the stimulation of established synapses to something more complex than a single spike.

5.5 Summary

Synaptic positions derived from distributions of 20-80% rise times of somatically recorded EPSPs indicate that synaptic contacts from neurons in the same or adjacent barrel-related column in the rat barrel cortex are preferentially made on basal and apical oblique dendrites of layer V IB pyramidal neurons. Whereas it is possible that the sampling of only the first arriving EPSPs in cases with multiple input is biased towards perisomatically located contacts with shorter delay-to-onset of the somatic depolarization, the derived distributions are in good agreement with experimentally determined distributions of synapses made by pyramidal neurons from layers III and V (see below). The calculated increase in synaptic distance with increasing rise time of the somatically recorded EPSPs of 90 μ m/ms is smaller than the 200 μ m/ms reported by Williams & Stuart (2000), however their experiments were carried out with a current injection waveform simulating

the synaptic input. This does not affect the integration properties of the neuronal membrane (and thus the time course of the EPSPs) in the same manner as a synaptically induced EPSP, since there is no local conductance change due to the opening of synaptic channels following a current injection.

Synapses formed by pairs connected pairs of layer V IB neurons have been shown to be predominantly located on the basal (~63%) and apical oblique dendrites (~27%) for pairs of closely spaced neighbouring cells (Markram et. al, 1997), whereas pairs of more separated layer V neurons form the more synapses on the apical oblique dendrites (Thomson and Deuchars, 1997). The connectivity of the stimulation sites simulated in the model were based on averaged population rise times (and thus synapse distributions) from experimentally induced responses by stimulation of either all intra- or transcolumnar layer V sites. Thus the similarities between synapse distributions on basal and apical oblique dendrites for stimulation sites in intra- and transcolumnar layer V is expected (tables 5.4 and 5.5), as the intracolumnar population contain more separated than nearest neighbour pairs.

Layer III pyramidal neurons have been reported to form synapses primarily on the apical oblique dendrites of layer V IB cells followed by basal dendrites and with smaller numbers on the larger apical dendrites and least in the apical tuft (Thomson & Bannister, 1998). The model distribution of synapses from sources in intra- and transcolumnar layer II/III on basal (16% and 30% respectively), apical oblique dendrites (43% and 41% respectively) and medial apical dendrites (28% and 19% respectively), are in good agreement with this report, with a slightly large fraction of contacts from intracolumnar layer II/III on the medial apical dendrites.

No specific information on the preferred locations of synapses formed by layer IV or VI neurons in the barrel cortex exists, however axonal tracing studies have shown that both star pyramidal and spiny stellate neurons in the layer IV have longer descending axonal collaterals that primary form local arbors in layers V and VI, also in neighbouring barrel-related columns (Lübke et al., 2000). The restriction of synaptic contacts made from layer IV stimulation sites in the model to primarily basal and oblique apical dendrites, with a small fraction made on the proximal part of the medial dendrites, suggest that synaptic contacts formed by layer IV neurons on layer V IB pyramidal cells are supplied by the descending branches of their axons and not the local arborization in layer IV.

Pyramidal neurons in layer VI have axonal arbors ascending through layers VI and V, with the longest branches reaching lower layer IV (Zhang & Deschênes, 1997). These findings support the possible restriction of synapses formed by layer IV and VI neurons to primarily the basal and apical oblique dendrites of layer V IB pyramidal cells as found in the simulation studies.

Simulations of elicited EPSP amplitudes in the layer V IB neuron model using single presynaptic spikes to activate a number of realistically distributed synapses showed good agreement with the experimental data (Schubert et al., 2001) for inputs originating from a number of layers. No significant differences between experiment and simulation for stimulations in intra- and transcolumnar layers II/III and VI, as well as the transcolumnar input from layer V. The model failed to capture the action potential inducing inputs from intracolumnar V, as well as the more distributed amplitudes and action potential inducing inputs from layer IV stimulation sites.

Inspection of the raw experimental data showed stimulations in intracolumnar layer V leading to AP firing always originated from sites within the neighbouring columns of the stimulation grid maximally 2-3 rows higher than the soma of the recorded neuron. This raises the possibility that closely spaced layer V neurons are more strongly connected than more distal pairs, or that the large fraction of synapses formed on basal dendrites (Markram et al., 1997) possibly lead to a better suited situation for inducing action potentials, as the shorter and more restricted propagation path to the soma would most likely result in less attenuation of the EPSPs. The possibility that the action potentials result from burst firing in presynaptic neurons seem unlikely, since no action potential firing was induced by stimuli more distal from the neuron or from sites in the neighbouring column.

The larger amplitude EPSPs and action potential inducing activations induced by stimulations in layer IV have previously been suggested to result from activation of groups of layer IV neurons recurrently exciting each other via the extensive local connections in the granular layer (Schubert et al., 2001). Additionally both spiny stellate cells and (star) pyramidal neurons have been reported capable of firing bursts of action potentials in response to stimulation by local uncaging of glutamate (Schubert et al., 2003). Further investigations involving dual recordings of synaptically coupled neurons in layer IV and V of the barrel cortex are necessary to determine the exact cause of the wider distribution of

EPSPs recorded in the layer V pyramidal cells after stimulating presynaptic sites in layer IV.

Whereas the amplitudes of the simulated EPSPs in the model were in good agreement with the experiment apart from the described exceptions, the calculated EPSP integrals were much smaller than those determined from the experimental recordings (Schubert et al., 2001). As the standard deviation of the integrals was more affected by this discrepancy, this effect is likely more pronounced for large EPSPs. This may indicate that the previously reported estimate of the NMDA glutamate receptor mediated current contribution to the integrals of being much smaller than 20% at membrane potentials lower than -60 mV (Markram et al., 1997) is too low, since larger depolarizations decrease the voltage dependent magnesium ion block of the receptor channel. This discrepancy could possible stem from the age difference of the rats used in the dual recording experiment (postnatal days 14-16) and the mapping experiment using release of caged glutamate (postnatal days 18-22), as the NMDA receptor mediated currents have been shown to increase until a peak level at the end of the third postnatal week (Glazewski et al., 1995).

6 Discussion

The results presented in this thesis, based on investigations using a combination of detailed neuronal modelling and analysis of data from previous experimental work, indicate a stereotyped synaptic target selection for neurons in the local microcircuitry of the rat barrel cortex. In spite of their long apical dendrites reaching all the way to the superficial layers of the cortex, layer V intrinsically bursting pyramidal neurons are inferred to primarily receive synaptic inputs on the basal and apical oblique dendrites from local neurons in all other cortical layers, as well as from populations in the neighbouring barrel-related columns.

Only pyramidal neurons in layer II/III appear to form a significant fraction of their synaptic contacts on the more distal parts of the apical dendrites of layer V IB pyramidal cells, but even for such pairs of connected neurons, the majority of synaptic input appears to be located onto the more proximal dendritic branches. The similarities in synaptic target selection for neurons forming intra- and transcolumnar connections indicate that stimulus properties integrated across columnar borders are involved in the same types of information processing as input originating from the local barrel-related column.

6.1 The use of complex models and databasing approaches

Detailed neuronal models based on experimental data have been used successfully to explore many complex interactions in a medium more easily manipulated than an actual experimental preparation, spanning from investigations of the importance of the complex morphological structures of different cell types, the impact of the differential distribution of membrane properties and conductances to interactions of detailed neuronal models in complex circuits and networks (De Schutter & Bower, 1994a; Destexhe et al., 1996; London & Segev, 2001; Korogod & Kulagina, 1998; Mainen et al., 1995; Mainen & Sejnowski, 1996; Stuart & Spruston, 1998). While detailed experimentally constrained models can pave the way for many insights, it is imperative that the quality or realistic behaviour of an implemented model is established to ensure the quality of the information gleaned from the simulations. Since the complex models provide the modeller with a multitude of parameters for fitting a selected experimental dataset, it is in principle possible to reproduce any kind of behaviour, given enough time to tune these parameters. Therefore it is critical that the models are not only initially constrained by experimental data, but that they are also subsequently tested against further experimental data without further adjustments, to ensure the generality of the model (De Schutter & Steuber, 2001).

The implemented detailed layer V pyramidal neuron model used for investigations of local connectivity in the rat barrel cortex in this thesis fulfils these criteria. While the model was originally tuned to fit the experimental I-V characterizations from a current injection protocol recorded from the neuron providing the reconstructed morphology used in the model (Schubert et al., 2001), the model was also shown to produce realistic responses to other simulated conditions in agreement with reports in the literature. This involved both simple tests such as the response to increased amplitude of depolarizing injection current and increased density of the persistent sodium conductance g_{NaP} (Mantegazza et al., 1998; Zhu & Connors, 1999), but the model also displayed backpropagation of action potentials into the apical dendrite with propagation speeds and durations closely matching the experimentally described physiological behaviour (Stuart et al., 1997). Furthermore, the model proved useful for the investigation of direct responses to stimulation with a simulated UV-flash release of caged glutamate (Schubert et al., 2001), producing realistic responses and elucidating the effect of distance from the focal plane of the photolysis inducing UV-flash on the recorded depolarizing responses. Taken together, the ability to produce such a large variety of physiologically realistic behaviours indicate that the results obtained with the implemented model are likely to be physiologically relevant and not simply artificially constructed results.

With the heavy dependence of complex models on experimental data for constraints and testing, the usefulness of databases providing the modeller with easy access to organized datasets is evident. Contrary to other fields like genetics or brain mapping, where intensive databasing efforts has been facilitated by standardized methods or data formats (Chicurel, 2000), experimental data from electrophysiological studies are as diverse as the methods employed and the phenomena investigated, ranging from recordings of single ion channels to activities of larger neuronal populations in one or more brain structures. In spite of these inherent obstacles, several efforts have been made but have often been limited to narrower aspects of the field (Ascoli et al., 2001; Cannon et al., 1998; Gonzalez et al., 2001).

Central collections of data in a standardized and easily accessible format could benefit modeller and experimentalist alike, not only providing information for construction of neurons but also a reference for comparison of experimental results with those of other investigators. This would be particularly useful if such collections were provided with descriptions of the experimental protocols used to obtain them (Gardner et al, 2001).

As was seen through the use of information extracted from the CoCoDat database for implementation of a layer V IB neuron model, the range of information required on specific neuron types or specific aspects of these exceeds by far the available data. As an example, even complete kinetic descriptions of the most predominant types of conductances is often not available, reflecting both the lack of systematic experimental mapping of neuron properties as well as the need for several data collators in order to populate a database in a reasonable amount of time. Some efforts into detailed quantitative characterization of neuron subtypes on several levels of description are being undertaken (Gupta et al., 2000), and could possibly pave the way for more systematic investigations. Given that such efforts come to fruition, attempts to generate standardized descriptions of model components like synaptic and voltage gated channels in the XML based mark-up language NeuroML (Goddard et al., 2001) would have an excellent source for "plug-and-play" model components, thus facilitating e.g. implementation of representative models for use in network simulations where the individual neuron must not necessarily be specifically tuned to reproduce the results of a specific experimentally characterized cell.

The implementation and testing of detailed neuron models can in part fill parts of the need for a larger amount of detailed information for databasing. Through the adaptation and adjustment of incomplete descriptions of e.g. ionic conductance kinetics followed by testing against data on whole cell behaviour, the models can attempt to "fill the holes" by providing tested descriptions that in conjunction with other well established descriptions produce realistic behaviour of the models. Ultimately, such predicted characteristics should be tested experimentally to validate or reject the model predictions, but starting with a better defined question or scope for the experimental study should also greatly facilitate the acquisition of useful data.

6.2 Functional implications of polarized synaptic target selection on the dendrites of layer V IB pyramidal neurons

The integration of distal and proximal dendritic inputs has been studied experimentally by a combination of proximal and distal current injections to layer V pyramidal neurons in slice preparations (Larkum et al., 1999; Berger & Lüscher, 2003). These studies show that weaker distal dendritic inputs in combination with somatic stimuli strong enough to elicit a single backpropagating action potential at the soma can lead to burst firing and the generation of dendritic calcium plateau potentials. Due to the attenuation of distal dendritic inputs during propagation to the soma of the neuron, smaller distal depolarizations are not alone sufficient to induce action potential firing, but the depolarization induced by the back-propagating action potential lowers the threshold for initiation of a dendritic calcium spike. This lasting dendritic potential can in turn cause further axo-somatic depolarization and shift the neuron into a burst-firing mode. Since this coupling is only effective when the distal dendritic stimulation is initiated within a time window of 3-7 ms following the initial backpropagating action potential, this coupling provides a mechanism for coincidence detection and association of perisomatic and distal dendritic inputs (Larkum et al., 1999; Berger & Lüscher, 2003). Further studies showed that depolarizing or hyperpolarizing inputs to the region of the apical oblique dendrites (mimicking excitatory and inhibitory synaptic inputs) can either enhance or abolish the coupling leading to burst firing (Larkum et al., 2001). These results establish that the position and sequence activation of synaptic contacts forming the local microcircuitry of the cortical columns will play a large role in determining the response of the neuron to the combinations of dendritic inputs received in the awake behaving animal.

The findings presented in this thesis indicate that local connections from neurons in the barrel cortex forming intra- and transcolumnar connections with the layer V pyramidal neurons predominantly target the apical oblique and basal dendritic domains of the cell. These domains are characterized by a large specific membrane resistivity and low resting conductance densities of the hyperpolarization activated conductance g_h (Stuart & Spruston, 1998) leading to large time constant, which favors an extensive temporal integration of converging synaptic inputs.

Contrary to this, corticocortical feedback connections from higher cortical areas selectively terminate in the cortical layer I and have been shown to provide substantial input to dendrites in the apical tuft of layer V pyramidal neurons, providing a top-down modulation of ongoing behavior (Cauller, 1994; Cauller & Connors, 1995). The distal apical dendrites of the layer V pyramidal neurons are characterized by a low specific membrane resistance as well a large resting conductance level of g_h (Stuart & Spruston, 1998). This results in a small membrane time constant, which necessitates a higher degree of temporal correlation for the summation of synaptic inputs.

Combining this information on different characteristic intrinsic properties and connectivity patterns of layer V pyramidal neurons help elucidate their reported important roles in learning and modulation of ongoing sensory behavior (Cauller, 1995; Diamond et al., 1999). Input from local sources in the barrel cortex stemming from the thalamocortical projections of the whisker-to-barrel pathway primarily affects the proximal dendrites, where extensive temporal summation takes place and leads to ongoing action potential firing activity. Feedback connections from higher cortical areas forming synapses on the dendritic branches can modulate this ongoing activity by way of the coupling provided by the back-propagating action potentials, leading to increased burst firing in the layer V IB neurons. This increased bursting can either serve to modulate the active exploration of the animal through projections to subcortical structures or provide a "reward" signal signifying a behaviorally relevant input leading to plastic changes and learning (Cauller, 1995; Diamond, 1999).

As the exception to the rule, pyramidal neurons in layer II/III also appear to form a significant number of synaptic contacts of the distal apical dendrites of layer V IB cells. However the layer II/III pyramidal neurons also receive input from the feedback connections in layer I (Cauller, 1995; Cauller & Connors, 1994) and could thus be involved boosting this feedback signal in the local microcircuit through their reciprocal connections with layer V IB neurons (Thomson & Bannister, 1998; Thomson & Deuchars, 1997). Furthermore, the reciprocal connections between layer II/III and V pyramidal neurons in a "triangular arrangement" (II/III \rightarrow V \rightarrow II/III) has been suggested to be the foundation of the formation of coherent neuronal assemblies, forming the basis of distributed representations of "meanings" or memories in the cortex (Miller, 1996). Here the supragranular pyramidal neurons are thought to be the primary "storage" of information due to their larger densities of the NMDA glutamate receptor subtype implicated in long

term plasticity changes. Whereas the output from the layer II/III pyramidal is thought to be too weak to induce the plastic changes necessary to form stable assemblies of neurons with strengthened connections, their output is theorized to be boosted by way of interactions with the layer V pyramidal neurons, before it is fed back to other layer II/III neurons. The mechanism behind this could be the described coupling of distal and proximal dendritic domains in the layer V pyramidal neurons, making it necessary for the layer II/III pyramidal neurons to form synapses on the distal apical dendrites of the layer V pyramidal cell.

7 Summary

The mammalian neocortex consists of repeated structural and functional units known as *columns*, spanning the six layers of the isocortex. A column encompasses different types of neurons sharing common response properties to afferent inputs. The coding and processing of information in local *microcircuits* comprised of interconnected neurons from different populations define the functional properties of the columns, and can be regarded as the basis of information processing in the cortex. The barrel cortex of the rat, which receives sensory information from the facial whiskers of the animal, provides a useful model system for investigating the properties of microcircuits, since the columnar structure is well defined with each large barrel-related column receiving its primary input from one facial whisker.

In this thesis, the detailed distribution of connections formed by neuronal populations in the local microcircuitry onto the dendrites of large layer V intrinsically burst firing (IB) pyramidal cells of the rat barrel cortex was investigated. These neurons have large dendritic trees consisting of subdomains with different intrinsic properties, suggesting that the specific position of individual synapses on the dendrites could be critical. Layer V IB pyramidal neurons have been shown to play a critical role in learning processes as well as the modulation of ongoing behaviour in the awake animal.

Simulations and analysis of extensive datasets from mapping of functional connections between layer V IB neurons and local neuronal populations were carried out using an experimentally constrained single neuron model. Simulation results suggest that projections from neurons located in the local and neighbouring columns of the rat barrel cortex selectively target the basal and proximal apical dendritic domains of the layer V IB neurons.

Feedback connections from higher cortical areas critical in learning processes are known to selectively target the distal apical tufts of layer V pyramidal cells, thus the findings in this thesis suggest the existence of a functional segregation of the inputs to different dendritic subdomains correlated with the intrinsic properties of the dendritic compartments that they target.

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