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Shining New Light on Motoneurons
Characterization of Motoneuron Dendritic Spines
Using Light Microscopy and Novel Analytical Methods

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Supervisors: Prof. Gregory Funk
Prof. Mark B. Cannell
Assoc. Prof. Christian Soeller

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY IN PHYSIOLOGY,
UNIVERSITY OF AUCKLAND, 2009
Abstract

Dendritic spines are fundamental units of information processing within the nervous system, responsible for independent modulation of synaptic input to neurons. Filopodia, often morphologically indistinguishable from spines, are involved in formation of synapses during neuronal development. Despite the importance of these structures for neuronal function, no detailed study of their presence on motoneurons has yet been made. Here, the presence of spines on hypoglossal motoneurons (HMs) is described at three developmental stages: at P0–2 and P9–11, spines are present at an average density of $\sim 0.1$ spines/µm, but at P19 spine density becomes negligible. In P0–2 and P9–11, spines are non-uniformly distributed, occurring in clusters, and at lower density in the most proximal and distal regions to the soma than at intermediate regions. HM spines coincide with a decrease in cell input resistance, which reduces excitability during development. Thus one may speculate that these spines are involved in the formation of new synapses required to maintain adequate excitatory drive.

A major difficulty for the study of spines is their small size, which complicates measurement using optical methods. Here, I present a novel method for reconstructing spine morphology using geometric models based on a priori knowledge of spine structure. Tests of the technique using simulated data indicate that it has a resolving capability of up to 40 nm (limited by noise). The technique has been used to measure dendritic spines on HMs, showing that these structures have necks as small as 0.22 µm. For purely passive modulation of synaptic strength, spine necks need to be $< \sim 0.15$ µm. These data suggest that if modulation of synaptic input occurs, biochemical and/or active electrical processes are needed.

The methods developed in this Thesis, which have here been applied to HMs, are generally applicable to the study of spine morphology, and its effect on synaptic processing, in all classes of neurons.
Acknowledgements

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To my parents, Judith and Donald, whose unconditional love and support for my endeavours have provided the foundation on which this Thesis has been possible.
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List of Symbols and Abbreviations

$\lambda$ wavelength or electrotonic length

[ ] concentration

2-D two-dimensional

3-D three-dimensional

2P two-photon

2PM two-photon microscopy

5-HT 5-hydroxytryptamine

n-D n-dimensional

A2D analog-to-digital

AMPA $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionate

ATP adenosine triphosphate

B byte

BDNF brain derived neurotrophic factor

Ca$^{2+}$ calcium

C/A commissural/associational

CAD computer-aided design

CaMK Ca$^{2+}$/calmodulin-dependent protein kinase

CaMKII Ca$^{2+}$/calmodulin-dependent protein kinase II

CARS coherent anti-Stokes Raman scattering

cdf cumulative distribution function

CICR Ca$^{2+}$-induced Ca$^{2+}$-release

CLSM confocal laser scanning microscopy

CPA cyclopiazonic acid

CPU central processing unit

CR carriage-return
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<td>continuous wave</td>
<td></td>
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<tr>
<td>D2A</td>
<td>digital to analog</td>
<td></td>
</tr>
<tr>
<td>DAB</td>
<td>di-amino-benzidine</td>
<td></td>
</tr>
<tr>
<td>DAC</td>
<td>digital-to-analog converter</td>
<td></td>
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<td>1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate</td>
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<tr>
<td>DIV</td>
<td>days in vitro</td>
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<tr>
<td>E</td>
<td>embryonic days old</td>
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</tr>
<tr>
<td>EGFP</td>
<td>enhanced green fluorescent protein</td>
<td></td>
</tr>
<tr>
<td>EGTA</td>
<td>ethylene glycol tetraacetic acid</td>
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<td>EM</td>
<td>electron microscope</td>
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<td>EPSC</td>
<td>excitatory post-synaptic current</td>
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<td>excitatory post-synaptic Ca(^{2+}) transient</td>
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<tr>
<td>EPSP</td>
<td>excitatory post-synaptic potential</td>
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<td>fAHP</td>
<td>fast after hyperpolarisation</td>
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<td>FD</td>
<td>fluorescein dextran</td>
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<td>fs</td>
<td>femtoseconds</td>
<td></td>
</tr>
<tr>
<td>FWHM</td>
<td>full-width at half-maximum</td>
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</tr>
<tr>
<td>GABA</td>
<td>(\gamma)-amino-butyric acid</td>
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</tr>
<tr>
<td>GG</td>
<td>genioglossal</td>
<td></td>
</tr>
<tr>
<td>GluR</td>
<td>glutamate receptor</td>
<td></td>
</tr>
<tr>
<td>GUI</td>
<td>graphical user interface</td>
<td></td>
</tr>
<tr>
<td>HFO</td>
<td>high frequency oscillation</td>
<td></td>
</tr>
<tr>
<td>HM</td>
<td>hypoglossal motoneuron</td>
<td></td>
</tr>
<tr>
<td>Hn</td>
<td>hypoglossal nucleus</td>
<td></td>
</tr>
<tr>
<td>HRP</td>
<td>horse radish peroxidase</td>
<td></td>
</tr>
<tr>
<td>HVA</td>
<td>high voltage-activated</td>
<td></td>
</tr>
<tr>
<td>Hz</td>
<td>hertz (cycles per second)</td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>infra-red</td>
<td></td>
</tr>
<tr>
<td>IP(_3)R</td>
<td>inositol-1,4,5-trisphosphate receptor</td>
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</tbody>
</table>
IPSP  inhibitory post-synaptic potential
IPSC  inhibitory post-synaptic current
ISR  interrupt service routine
K⁺  potassium
LSM  laser scanning microscope or microscopy
LTD  long-term depression
LTP  long-term potentiation
LUT  lookup table
LVA  low voltage-activated
mAHP  medium-duration after hyperpolarisation
MAPK  mitogen-activated protein kinase
MB  megabyte
mEPSP  miniature excitatory post-synaptic potential
mGluR  metabotropic glutamate receptor
MN  motoneuron
mW  milliwatts
Na⁺  sodium
NA  numerical aperture
NA  noradrenaline or noradrenergic
NCX  Na⁺-Ca²⁺ exchanger
NK₁R  neurokinin-1 receptor
nm  nanometer
NMDA  N-methyl-D-aspartate
op-amp  operational amplifier
OSA  obstructive sleep apnoea
P₂XR₅  purinergic-2 receptors
P  post-natal days old
PAGFP  photoactivatable green fluorescent protein
PBS  phosphate-buffered saline
PC  personal computer
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PMCA</td>
<td>plasma membrane Ca(^{2+})-ATPase</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier tube</td>
</tr>
<tr>
<td>preMNs</td>
<td>pre-motoneurons</td>
</tr>
<tr>
<td>PSD</td>
<td>post-synaptic density</td>
</tr>
<tr>
<td>PSF</td>
<td>point-spread function</td>
</tr>
<tr>
<td>PSP</td>
<td>post-synaptic potential</td>
</tr>
<tr>
<td>(R_m)</td>
<td>specific membrane resistance (in k(\Omega) (\cdot) cm(^2))</td>
</tr>
<tr>
<td>(R_N)</td>
<td>input resistance (in (\Omega))</td>
</tr>
<tr>
<td>REM</td>
<td>rapid eye movement</td>
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<tr>
<td>RyR</td>
<td>ryanodine receptor</td>
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<tr>
<td>S</td>
<td>Siemen</td>
</tr>
<tr>
<td>SHG</td>
<td>second harmonic generation</td>
</tr>
<tr>
<td>SIDS</td>
<td>sudden infant death syndrome</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SER</td>
<td>smooth endoplasmic reticulum</td>
</tr>
<tr>
<td>SERCA</td>
<td>sarco/endoplasmic reticulum Ca(^{2+}) ATPase</td>
</tr>
<tr>
<td>S/N</td>
<td>signal-to-noise ratio</td>
</tr>
<tr>
<td>SP</td>
<td>substance P</td>
</tr>
<tr>
<td>SP</td>
<td>short-pass</td>
</tr>
<tr>
<td>TEA</td>
<td>tetraethylammonium</td>
</tr>
<tr>
<td>TDA</td>
<td>transient dendritic appendage</td>
</tr>
<tr>
<td>TLM</td>
<td>transmitted light microscopy</td>
</tr>
<tr>
<td>TRH</td>
<td>thyrotropin-releasing hormone</td>
</tr>
<tr>
<td>TTX</td>
<td>tetrodotoxin</td>
</tr>
<tr>
<td>UV</td>
<td>ultra-violet</td>
</tr>
<tr>
<td>VSIC</td>
<td>voltage-sensitive ion channel</td>
</tr>
<tr>
<td>VSCC</td>
<td>voltage-sensitive calcium channel</td>
</tr>
<tr>
<td>VB6</td>
<td>Visual Basic 6</td>
</tr>
<tr>
<td>VSG</td>
<td>ventral swallowing group</td>
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<tr>
<td>WF</td>
<td>wide-field</td>
</tr>
<tr>
<td>XII</td>
<td>twelfth cranial (hypoglossal)</td>
</tr>
</tbody>
</table>
List of Suppliers and Manufacturers

MBF  MBF Biosciences, Williston, VT, USA

Newport  Newport Corporation, Irvine, CA, USA

GSI  GSI Lumonics, Billerica, MA, USA

Probes  Molecular Probes, Invitrogen, Eugene, OR, USA

Sigma  Sigma-Aldrich, St Louis, MO, USA

Zeiss  Carl Zeiss Ltd., Oberkochen, Germany