Pacemaking in Mouse Locus Coeruleus Neurons: Electrophysiological Properties, Role of Mitochondria and Development

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BSc in Pharmacy and Master in Biochemistry

Thesis submitted in fulfilment of the requirements for obtain the degree of Doctor of Philosophy in Human Physiology

School of Biomedical Sciences and Pharmacy

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September 2010
Statement of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Ramatis Birnfeld de Oliveira
Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of my thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications.

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<th>Paper</th>
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<td>de Oliveira, R. B.; Graham, Brett; Howlett, Marcus C.H.; Gravina, Fernanda S.; Oliveira, Max W.S.; Imtiaz, Mohammad S.; Callister, Robert J.; Lim, Rebecca; Brichta, Alan M.; van Helden, Dirk F.</td>
<td>Ketamine anesthesia helps preserve neuronal viability. Journal of Neuroscience Methods, v. 189, p. 230-232, 2010. DOI: 10.1016/j.jneumeth.2010.03.029</td>
<td>80% of the total Dirk F. van Helden</td>
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<td>de Oliveira, Ramatis B.; Gravina, Fernanda S.; Brichta, Alan M.; Callister, Robert J.; van Helden, Dirk F.</td>
<td>Effects of mitochondrial disruption on ionic currents and pacemaking in neurons of the locus coeruleus. Manuscript under review in “Journal of Physiology”.</td>
<td>90% of the total Dirk F. van Helden</td>
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Acknowledgments

I first would like to thank the “invisible mystery” that is out there somehow guiding us to amazing opportunities every day. One of these opportunities crossed my way and here I am after 4 years in the other side of the world finalizing my PhD thesis.

My beloved wife Fernanda is the first living one that I would like to thank, not only for her endeavours together with me in this journey but also for everything that she represents to me. Infinite love and affection are just words that cannot describe my feelings for this “little person”.

I also would like to thank my supervisor Dirk for having accepted us into his lab and supported us during all this time. More than a supervisor I have a good friend.

Marcus Howlett also deserves a big thank you for all the support that he gave me and for all his patience and comprehension with my “recently learned” English. Bob Callister also deserves a big thank for his help in reviewing the manuscripts.

Someone that also needs to be thanked is my good friend and past supervisor Jose Claudio, if was not for his outstanding comprehension and wisdom, I would never have been able to get here in Australia in time. In the same way I would like to thank a guy that is more than a friend, Daniel Gelain, for introducing us to Dirk and for all these years of friendship.

I also would like to thank my family (old and new members) for all their support and unconditional love. I guess if was not for the love, education and moral values received from them I would not be as happy as I am and for sure would not love so much to live.
I think they taught me the most important value in life that is that life never ends and every minute is important to do something in retribution to the things that we receive.

A big thanks also goes to my good friend Peter Dosen for all his technical support and friendship during all these 4 years.

Everybody that somehow helped me to carry on during this PhD period please also feel thanked.
Table of Contents

List of Abbreviations .......................................................................................................................... 10
Publications ........................................................................................................................................... 17
Chapter 1 - General Introduction........................................................................................................ 20
  1.1. The Locus coeruleus .................................................................................................................. 21
  1.2. Pacemaker Mechanisms .......................................................................................................... 26
    1.2.1 Cardiac Pacemaker Model (Plasmalemmal Pacemaking) ...................................................... 26
    1.2.2 Intracellular Stores Model .................................................................................................. 27
  1.3. Brain Pacemaking ..................................................................................................................... 29
    1.3.1. Pacemaking in Locus Coeruleus ...................................................................................... 29
    1.3.2. Pacemaking in other Brain Areas ...................................................................................... 33
  1.4. Mitochondria ............................................................................................................................ 36
    1.4.1. General information .......................................................................................................... 36
    1.4.2. Role of Mitochondria in Cellular Pacemaking .................................................................. 37
  1.5. Brain development and pacemaker mechanisms ....................................................................... 39
Chapter 2 - Methods ............................................................................................................................ 41
  2.1. Preparation of brain slices ....................................................................................................... 42
  2.2. Preparation of fresh dissociated LC neurons ............................................................................ 43
  2.3. Electrophysiology .................................................................................................................... 43
  2.4. Solutions and pharmacology ................................................................................................... 44
  2.5. Acquisition and analysis ........................................................................................................... 47
  2.6. Cytosolic Ca\(^{2+}\) and Ψm measurement .................................................................................. 48
  2.7. Immunohistochemistry ............................................................................................................. 49
  2.8. Predicted net interspike interval pacemaker current ................................................................ 50
Chapter 3 - Ketamine anaesthesia helps preserve neuronal viability .................................................. 51
5.3.2. Outward currents in infant and adult mice .................................................. 92

5.3.3. Differences in inward currents during development................................. 97

5.4. Discussion ...................................................................................................... 101

5.5. Ongoing and future studies ............................................................................. 105

Chapter 6 - Effects of mitochondrial disruption on ionic currents and pacemaking in neurons of the locus coeruleus ................................................................. 106

6.1. Abstract .......................................................................................................... 107

6.2. Introduction .................................................................................................... 108

6.3. Results ............................................................................................................ 110

  6.3.1. Effect of CCCP on spontaneous LC firing, Ψm and voltage-dependent currents .............................................................................................................. 110

  6.3.2. CCCP-induced outward conductance is dependent on Ca^{2+} entry but independent on [Ca^{2+}]_c ....................................................................................... 115

  6.3.3. Calcium channel modulation and Ψm ....................................................... 118

  6.3.4. The CCCP-induced outward current is partially inhibited by apamin........ 120

6.4. Discussion ...................................................................................................... 123

6.5. Ongoing and future studies ............................................................................. 126

6.6. Supplementary Results ................................................................................... 128

  6.6.1. Effect of High BAPTA on the CCCP-induced hyperpolarization .......... 128

Chapter 7 – General Discussion ........................................................................ 129

Perspectives .......................................................................................................... 135

References ............................................................................................................. 137
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ACSF</td>
<td>artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AHP</td>
<td>after hyperpolarization</td>
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<tr>
<td>AP</td>
<td>Action potential</td>
</tr>
<tr>
<td>CCCP</td>
<td>carbonyl cyanide m-chlorophenylhydrazone</td>
</tr>
<tr>
<td>CICR</td>
<td>Ca$^{2+}$-induced Ca$^{2+}$ release</td>
</tr>
<tr>
<td>ChTx</td>
<td>Charybdotoxin</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EGTA</td>
<td>Ethylene glycol tetra acetic acid</td>
</tr>
<tr>
<td>FCCP</td>
<td>p-trifluoromethoxyphenylhydrazone</td>
</tr>
<tr>
<td>$I_A$</td>
<td>fast transient K$^+$ current ($I_A$)</td>
</tr>
<tr>
<td>$I_{Cl}$</td>
<td>chloride current</td>
</tr>
<tr>
<td>$I_H$</td>
<td>hyperpolarization-activated current</td>
</tr>
<tr>
<td>$I_{K-Ca}$</td>
<td>Ca$^{2+}$-activated K$^+$ current</td>
</tr>
<tr>
<td>$K_{Ca}$</td>
<td>Ca$^{2+}$-activated K$^+$ channel</td>
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$K_{IR}$ - $K^+$ inward rectifier current

LC – Locus coeruleus

MC – Membrane capacitance

MTC – Motor cortex

MMP – Mitochondrial membrane potential

NMDG - N-methyl-D-glucamine chloride

MNCE - Mitochondrial Na$^+$/Ca$^{2+}$ exchanger

PBS - Physiological buffer solution

PD – Parkinson’s diseases

$R_{IN}$ – input resistance

SN – Substantia nigra

TEA – Tetraethylammonium chloride

TH – Tyrosine hydroxylase

TTX – Tetrodotoxin

$V_m$ – membrane potential

4-AP - 4-Aminopyridine

$[Ca^{2+}]_c$ - cytosolic Ca$^{2+}$ concentration

$\Psi_m$ - Mitochondrial membrane potent
Abstract

This thesis presents studies on the electrophysiological properties of Locus coeruleus (LC), a group of noradrenergic neurons present in the brain stem pons. The specific interest in this neuronal population came from the fact that this neuronal population accounts for the largest noradrenergic nucleus present in the brain, providing noradrenaline input to virtually all cerebral regions and involved in controlling or modulating many types of behaviours. Another important feature is the recent “rediscovery” that LC neurons are involved in Parkinson’s disease (PD), where loss of activity and/or decrease in the neuronal population are thought to be a fundamental step to the progression and maintenance of this pathology.

LC neurons release many different types of neurotransmitters, noradrenaline, however, being the most studied. Regardless, of the neurotransmitter involved, release is controlled by intrinsic spontaneous firing activity, which is the result of a combination of pacemaker currents in balance with each other. These currents determine membrane depolarization and action potential initiation, and are responsible for adjusting the neuronal firing rate according to system needs. Even though the electrophysiological properties of LC neurons have been studied for decades, the mechanisms controlling and generating the pacemaker process need further investigation. As this process is a key feature underlying the influence exerted by LC neurons, a better understanding of this process is fundamental to determining how the brain functions and how impairment of LC activity may lead to neurological disorders. For example, it has been demonstrated in PD that along with the massive loss of Substantia Nigra (SN) neurons, the noradrenergic signalling provided by LC neurons to
the SN also suffers a massive impairment. Recently, it was suggested that noradrenaline exerts a neuronal protective effect against oxidative stress and inflammation. This is of great significance, as oxidative stress and inflammation are thought to be significantly involved in the development and maintenance of PD. These finding led to the proposal that impairment in the noradrenergic signalling provided by LC neurons to the SN could be a trigger in the generation and progression of PD.

All these facts inspired me to undertake detailed investigations into LC neuronal function. My aim was to first establish a solid understanding of the pacemaker process and then integrate this knowledge into a broader context. By choosing the mouse model this would allow future detailed studies using targeted mutants. I began by investigating the cellular basis of spontaneous firing of LC neurons with special emphasis on understanding the underlying pacemaker mechanisms, particularly those involved in action potential generation. In addition, I studied the effect of age and the possible involvement of mitochondria as a regulator of this process.

The first research chapter (see chapter 3) presents a methodological approach that was found to significantly improve the viability of LC neurons. This facilitated our electrophysiological studies and should be of general use for all those investigating brain neurons in vitro. LC neurons are medium-sized cells with large dendritic arbours. As a result, achieving proper voltage control in voltage clamp experiments in slice preparations is a challenge. It was found that the ketamine-based anaesthesia improved neuronal viability by substantially increasing the membrane input resistance and rate of success in voltage clamp experiments. This was not only found to be the case for LC neurons but was also confirmed in another neuronal population, namely hypoglossal motor neurons.
The second research chapter (chapter 4) characterizes the combination of pacemaker currents responsible for establishing depolarization during the interspike interval voltage range in mouse LC neurons. By using a series of voltage clamp approaches, I demonstrated that the composition of currents included a TTX-sensitive voltage-dependent Na$^+$ conductance, a high TEA-sensitive K$^+$ conductance, but no Ca$^{2+}$ conductance. Interestingly, this complement of currents is different to rat LC neurons, the most studied LC preparation, which exhibit an important persistent pacemaker Ca$^{2+}$ current close to the threshold for action potential initiation. A lack of a persistent Ca$^{2+}$ current in mice could represent a significant temporal change in all Ca$^{2+}$-dependent pathways giving a different perspective for cellular experiments carried out in the two species. This reminds us that direct comparisons between different animal species should be done with care.

The third research chapter (chapter 5) investigates developmental changes in LC pacemaker currents. The two basic conductances (i.e. Na$^+$ and K$^+$), responsible for controlling the membrane depolarization underwent changes during development. However, once again there was no evidence for Ca$^{2+}$ conductance in the pacemaker-interval voltage range in LC neurons, either infant or adult mice. Changes in the basic pacemaker currents were accompanied by other changes such as differences in TTX-insensitive spiking activity and the levels of baseline voltage noise. This chapter presents the first detailed report on the way development induces electrophysiological changes in mouse LC neurons.

The fourth paper (chapter 6) of this thesis presents an investigation into the possible participation of mitochondria as an active modulator of LC pacemaking. Aspects of mitochondria were investigated for two main reasons: 1) they act as significant Ca$^{2+}$
stores and are involved in controlling the intracellular Ca\(^{2+}\) dynamic in many different cells, hence they may impact on the pacemaking process of LC neurons; and 2) many neuropathologies such as PD have mitochondrial dysfunction as primary cause, thus the understanding of the role played by mitochondria in the pacemaking process could pave the way to discover new clinical approaches that may be beneficial at very early stages of the disease. Specifically, it was found that disturbance of mitochondrial metabolism activated Ca\(^{2+}\) channels that, in turn, activated K\(^+\) channels, which resulted in changes in the spontaneous firing activity of LC neurons. Two significant observations were made. The first was that Ca\(^{2+}\) channels activated by mitochondria were nifedipine sensitive, suggesting these were L-type Ca\(^{2+}\) channels. However, these voltage-dependent channels were activated at unusual hyperpolarized membrane potentials indicating an alternative pathway for their activation. The second observation was that activation of K\(^+\) channels involved Ca\(^{2+}\) entry but was independent of intracellular Ca\(^{2+}\) release. These findings are of significant interest since they demonstrate a pathway for K\(^+\) channel activation that is different than previously described by other investigators. Moreover, the fact that mitochondria may be involved in the pacemaker process, by not only buffering internal Ca\(^{2+}\) but also modulating ionic channels, indicates an exciting new perspective that could be applied to studies involving PD.

In summary, this thesis has characterized conductances responsible for controlling neuronal pacemaking in infant and adult mouse LC. Investigations of inhibiting mitochondrial function led to the proposal that mitochondria may serve as fine tune regulators of LC pacemaking. These results constitute a detailed description of fundamental electrophysiological properties of mouse LC neurons, which provide a foundation for future LC studies using mutant mice and thus providing new insights into mechanisms that control LC pacemaking. Going still further, the results presented here
can be applied to the study of the mechanisms underlying the progression of neurological diseases, such as PD, where there is direct involvement of LC neurons and their mitochondria.
Publications

The following publications have arisen from data presented in the present thesis:

Journal Articles


- de Oliveira, Ramatis B.; Gravina, Fernanda S.; Brichta, Alan M.; Callister, Robert J.; van Helden, Dirk F.. Effects of mitochondrial disruption on ionic currents and pacemaking in neurons of the locus coeruleus. Manucript under review in “Journal of Physiology”.

- de Oliveira, Ramatis B.; Gravina, Fernanda S.; Brichta, Alan M.; Callister, Robert J.; van Helden, Dirk F. Developmental changes in pacemaker currents in mouse locus coeruleus neurons. Manuscript under construction to be sent for publication.
Conference Abstracts


