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By seizures, glucocorticoids and microRNAs

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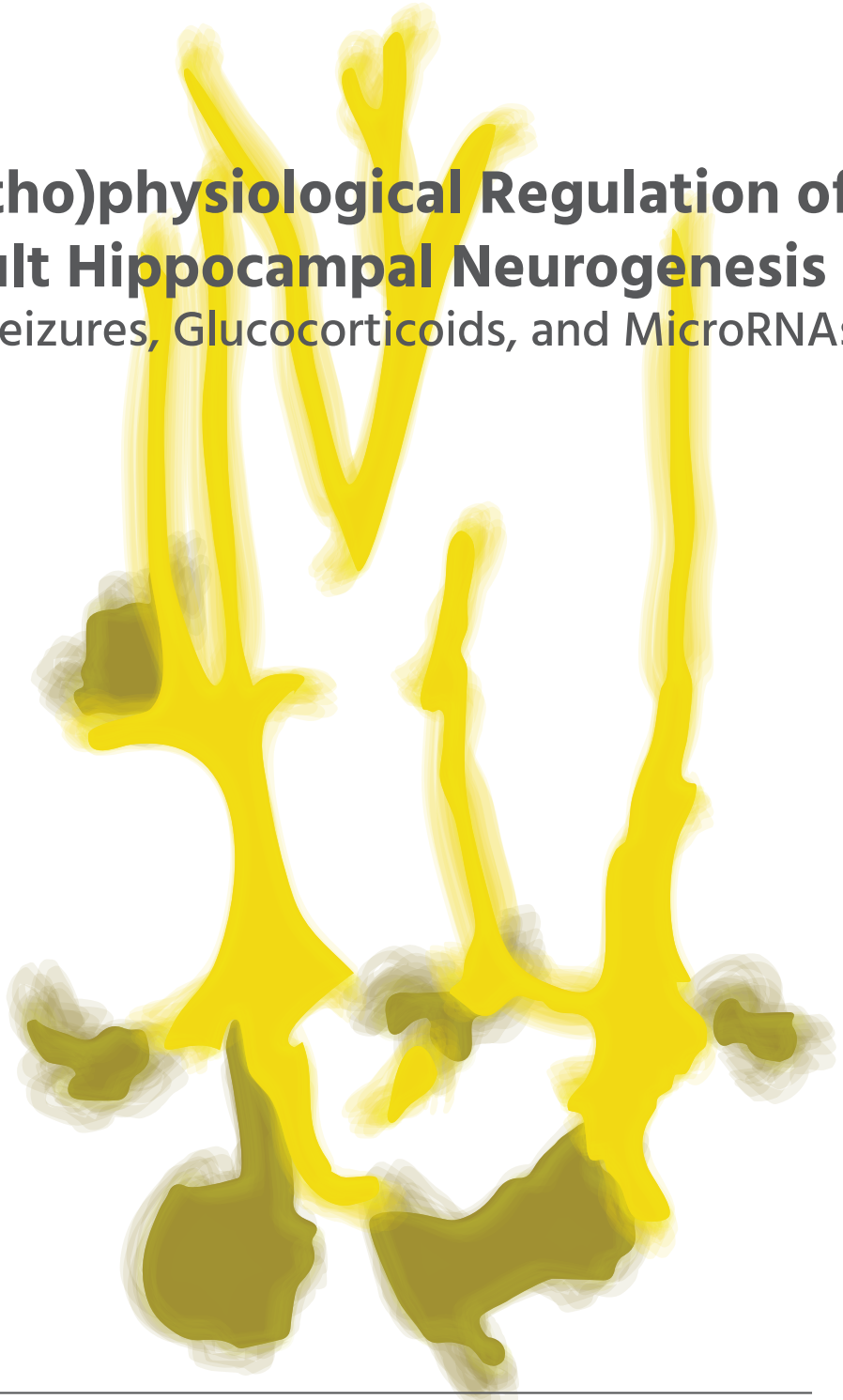

(Patho)physiological Regulation of Adult Hippocampal Neurogenesis
by Seizures, Glucocorticoids, and MicroRNAs

Pascal Bielefeld

(Patho)physiological regulation of adult hippocampal neurogenesis by seizures, glucocorticoids and microRNAs

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The studies in this thesis were performed at the department of Structural and Functional Plasticity of the Nervous System, Center for Neurosciences, University of Amsterdam (Chapter 1-6), the oncoproteomics laboratory, Cancer Center, Free University (Chapter 3), the Achucarro Basque Center for Neuroscience, University of the Basque Country, Spain (Chapter 2 and 5), and the Baylor College of Medicine, Texas Children's hospital, USA) (Chapter 2). I hereby declare that chapters 3 and 5 are shared projects performed in close collaboration with Marijn Schouten, and parts of this work have been used in his PhD thesis. From the work in these chapters, I have performed the majority of the *in vivo* experiments and analyses, while Marijn Schouten performed the majority of the *in vitro* and *in silico* studies. The studies in this thesis were funded by a NWO VIDI grant to C.P. Fitzsimons and the printing of this thesis was kindly supported by TSE systems GmbH, Germany, and Carlos Fitzsimons.

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Epigenetic Control of Hippocampal Stem Cells

Pascal Bielefeld
Paul J. Lucassen
Carlos P. Fitzsimons

Preface

In order to respond to changes in the environment, various levels of plasticity can help our brain to adapt successfully. This allows it to survive the challenges it is exposed to throughout life[1,2]. Plasticity happens at both the structural level, e.g. by adding new neurons to specific neurogenic regions of the brain, like the hippocampal Dentate Gyrus, a process referred to as Adult Hippocampal Neurogenesis (AHN), or e.g. by modifying the size of dendritic trees or the numbers of spines of preexisting neurons, but also at the functional level, by e.g. altering connectivity between neurons and changing synaptic plasticity[3]. Under physiological conditions, these processes are tightly regulated by various hormonal, environmental, and molecular factors[4–6].

Adult hippocampal neurogenesis comprises a complex cascade of events, starting with the activation of quiescent resident Neural Stem Cells (NSC), followed by asymmetric cell division, rendering a new stem cell and a daughter neural progenitor. These neural progenitors then amplify through symmetric divisions and undergo cell fate decisions, whereas some cells are depleted through apoptosis. The surviving neural progenitors can then differentiate into immature neurons, which over time, will mature and integrate into the pre-existing hippocampal network[2,7]. Each of these steps in this cascade requires complex and rapid changes in the molecular machinery, which usually comprise multiple levels of molecular control. Many of these regulatory layers involve the epigenetic machinery, including processes like DNA methylation and microRNA-mediated repression of gene expression[8,9].

When environmental challenges become more stringent, e.g. during severe stress exposure or in the case of pathological conditions like epilepsy, the structural and functional plasticity of the brain is generally insufficient and/or its regulation becomes aberrant or lost. This can result in the establishment of inappropriate neuronal connections and the faulty integration of e.g. unfit newborn neurons, termed aberrant AHN[10–12].

In order to understand how such pathologies can lead to a maladaptive plastic response, this thesis focuses on two different aspects. First, we aim to identify how epileptic seizures disturb the expression patterns of genes involved in the regulation of adult hippocampal neurogenesis, and how this may contribute to the aberrant hippocampal neurogenesis characteristics of the epileptic brain. Secondly, we focus on stress hormones, in particular glucocorticoids, and their role in regulating adult hippocampal neurogenesis under both physiological and pathological conditions.

Outline of this thesis

In **chapter 1**, I will discuss the consequences of epileptic seizures for the functionality of newborn hippocampal neurons and how seizure-induced changes could either contribute to the formation of an 'epileptic' hippocampal network, or alternatively might provide the brain with an intrinsic therapeutic mechanism, possibly counteracting the hyperactivation that is caused by initial epileptic seizures. To gain further insight in how epileptic seizures lead to aberrant neurogenesis, I provide an overview of the literature on the regulation of adult hippocampal neurogenesis by microRNAs and follow up with an overview of the seizure-induced changes in microRNA expression profiles before I discuss the effects this may have on cells at different stages of the neurogenic cascade.

In **chapter 2**, we describe a cross-laboratory standardized working protocol for the induction of epileptic seizures of variable intensity, using intrahippocampal administration of Kainic Acid (KA), and two different options for *in vivo* electrophysiological recordings. We identified a less severe status epilepticus-inducing KA dosage, which elicits electrophysiological responses as well. This protocol forms the basis for the experimental work carried out in **chapters 3 and 4**.

Chapter 3 describes a multi-omics approach to study the effects of KA-induced seizures on gene expression, protein expression, and microRNA profiles. This chapter provides both a working protocol and the obtained data set from our experiments. These multi-omic datasets in turn form the basis for the experiments carried out in **chapter 4**.

In **chapter 4** we focus on the effects of KA-induced seizures on different stages of the neurogenic cascade. We show that lower dosages of KA administered intrahippocampally induce a less severe Status Epilepticus, and that seizures of varying intensities have differential effects on AHN. We then explore the effect of lower-grade epileptic seizures on the neurogenic cascade and focus on neural stem cell fate. We show that non-convulsive epileptic seizures cause a shift in neural stem cell fate, which is different from the shift towards astrogenesis normally seen under severe status epilepticus conditions. Next, using the multi-omic datasets obtained in **chapter 3**, we investigate the role of two specific microRNAs, miR-124 and miR-137, in these altered cell fate decisions after epileptic seizures. We show that both miR-124 and miR-137 are involved in the shift in cell fate observed after KA-induced epileptic seizures.

In **chapter 5** we focus on the regulation of adult hippocampal neurogenesis by glucocorticoids as modulators of structural plasticity in the hippocampus. We show that deviations from the normal pulsatile glucocorticoid profile induce long-lasting changes in promoter methylation profiles, which in turn results in altered neural stem cell responses to subsequent glucocorticoid exposure. Furthermore, we describe how ageing affects the neurogenic capacity of the hippocampal stem cell pool, and address the role the glucocorticoid receptor plays herein. We found two populations of neural stem cells in the hippocampus; one that expresses the GR, and one that does not. These populations differ in their decay kinetics over time, indicating a protective role for the GR in maintaining neurogenic capacity with advancing age.

In **chapter 6** we provide a general discussion on the topics in this thesis and provide future directions.

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