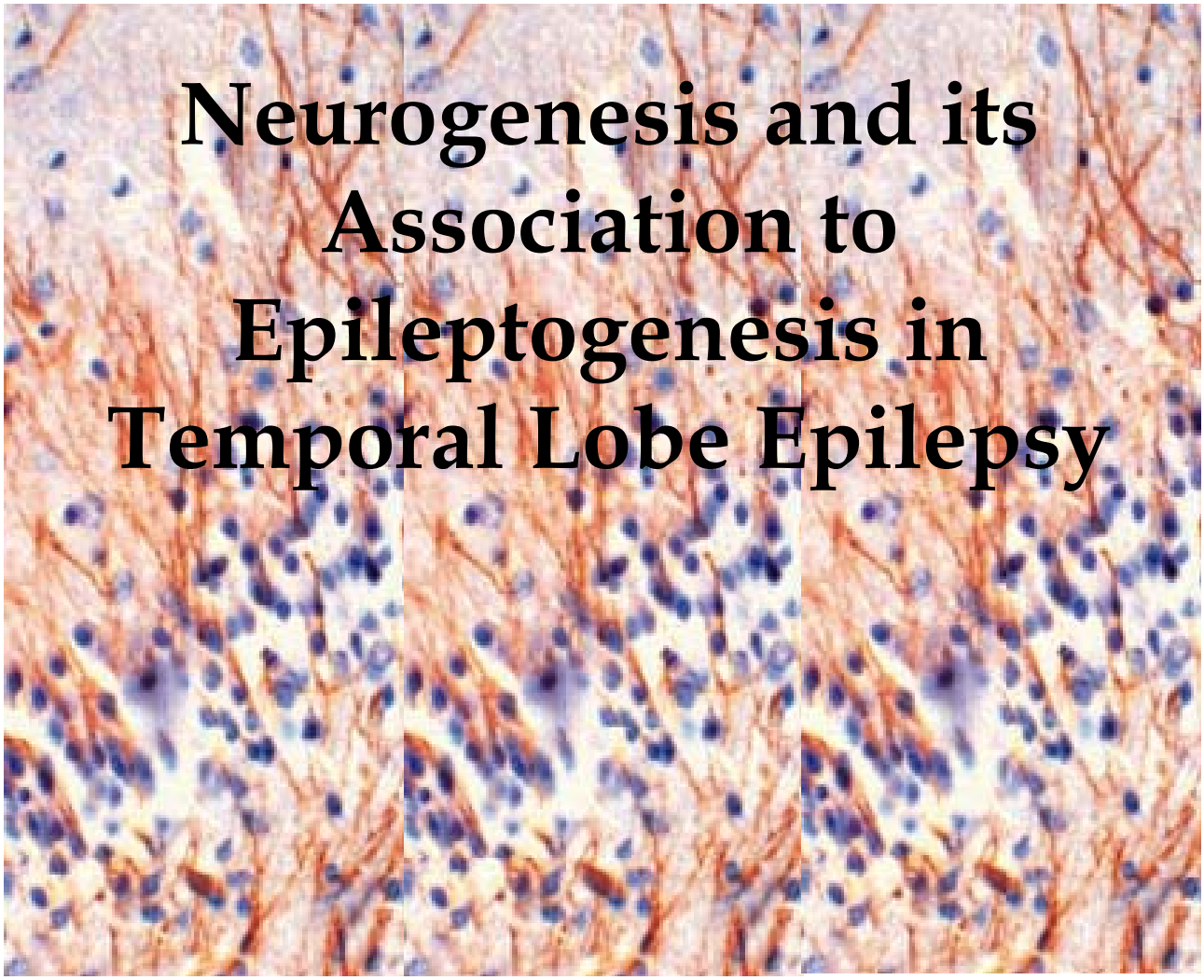

Neurogenesis and its Association to Epileptogenesis in Temporal Lobe Epilepsy

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Cover page: Figure adapted from Siebzehnrubl FA. and Blumcke I., 2008

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Abstract

Temporal lobe epilepsy (TLE) is the most common partial epilepsy[11]. Therefore, it is crucial to understand the mechanisms that contribute to the development of chronic TLE. It was generally believed that neurogenesis took place only during brain development, until in recent years proliferating cells were identified in the hippocampal dentate gyrus and olfactory bulb, providing compelling evidence that neurogenesis still occurs during adult life. Interestingly, studies have shown that seizure activity affects cell proliferation in animal models, proposing that it may potentially be involved in epileptogenesis. As yet, several studies have addressed this issue, yielding contradicting results. It has been demonstrated in animal seizure models that during the acute phase, neurogenesis briefly increases, subsequently decreasing in the chronic phase. Accumulating evidence suggests that neurogenesis does not play an essential role in epileptogenesis in adult rodents. Interestingly, age and/or disease related changes have been implicated in modifying the hippocampal environment so it no longer supports or promotes neurogenesis. Further, studies have shown that status epilepticus induction elicits a different response in the immature hippocampus, implying that in pups aberrant neurogenesis may play a more important role in epileptogenesis. Therefore, it should be determined whether neurogenesis is aberrant following seizures in rat pups and if it has long-term effects on the development of chronic seizures.

Chapter 1

Introduction to Epilepsy and Temporal Lobe Epilepsy

1.1 The Basics of Epilepsy

Epilepsy is a common neurological disorder characterized by a state of periodic and spontaneous seizures, which are periods of abnormal and simultaneous firing of a neuronal population. It is estimated to affect about 50 million people worldwide with 55/100.000 new cases appearing each year[1-3]. This complex disorder encloses more than 40 types of epilepsies, which differ in clinical manifestations and response to treatment. Therefore, epilepsy should not be defined as a disease, but rather as an epileptic syndrome.

It is not bewildering that the aetiology of epilepsy is so complex. Seizures are thought to be the consequence of an imbalance between inhibition and excitation resulting in the hyperexcitability of neuronal populations. This process can originate in a specific neuronal population, which is called partial epilepsy, or simultaneously in several brain areas, which is known as generalized epilepsy[4]. However, the neurobiological mechanisms that underlie this imbalance are still unclear. It is most likely that several genetic, molecular and cellular mechanisms are involved in the aetiology of epilepsy.

Since the epileptic syndrome is very heterogeneous, it is probable that different mechanisms contribute to the development of seizures. They can be secondary to an initial brain insult such as stroke, tumors, head injury, status epilepticus (SE) and febrile seizures, in which case they are called symptomatic. Idiopathic epilepsy, on the contrary, has no apparent initial insult. Recent studies have shown that genes may play a strong role in the development of idiopathic, as well as symptomatic epilepsies[5, 6]. Furthermore, research suggests that certain genes predispose individuals to develop symptomatic epilepsies, such as temporal lobe epilepsy (TLE) the most common form of epilepsy, which will be addressed in the next section[7-10]. However, how genes and an initial brain injury lead to the development of recurrent and spontaneous seizures in the temporal lobe has yet to be clarified.

1.2 The Pathophysiology and Epileptogenesis of Temporal Lobe Epilepsy

TLE is the most common partial epilepsy. Studies have shown that up to 53% of the TLE cases had febrile seizures as a child, suggesting this as an important risk factor. Moreover, 40% of the TLE patients will develop refractory epilepsy[7, 11, 12]. Therefore, understanding the mechanisms that lead to the development of chronic TLE is crucial to improve the existing treatment.

The recurrent seizures originate from the medial temporal lobe after an acute brain insult followed by a latent period, during which cellular and molecular changes are thought to occur. This period can last up to decades, before it eventually leads to a chronic period, which is defined by recurrent seizures *i.e.* status epilepticus (SE). Seizures and SE are different from each other as the first induces damage while the other does not. As the extent of the latent period can vary, some have postulated that a second hit may be necessary to trigger chronic epilepsy (see figure 1.1). This second hit could be an environmental factor or time-dependent gene expression[3, 13]. This difference might also result from the combined effect of differential gene expression and initial insults, which will lead to a variety of cellular and molecular changes that on their turn determine the extent of the latent period.

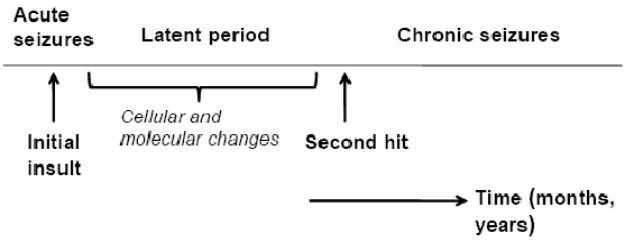

Figure 1.1: Schematic timeline depicting the events preceding the development of chronic temporal lobe epilepsy.

After the acute insult, several pathophysiological changes take place in the epileptic temporal lobe, particularly in the hippocampus. This may be because the hippocampus, amygdala and piriform-cortex are more susceptible to seizure- inducing brain insults[14]. The hippocampal circuitry is fundamental for information processing, as disorders affecting this brain region result in impaired cognitive functions. The hippocampus consists of three main regions, *viz.* the dentate gyrus, which has primarily granule cells, and the CA3 and CA1 areas both containing pyramidal neurons. These regions compose a trisynaptic circuit which is consists of three glutamatergic synapses. First, the perforant path axons from the entorhinal cortex project to the granule cells in the outer molecular layer (ML), next the mossy fiber axons of the granule cells innervate the pyramidal cells in the CA3 area, and the Schaffer collateral axons of these cells project to the pyramidal cells in the CA1 area (see figure 1.2). Further, mossy fibers and the perforant path also innervate GABAergic neurons and glutamatergic mossy cells in the hilus, which project to granule cells[15].

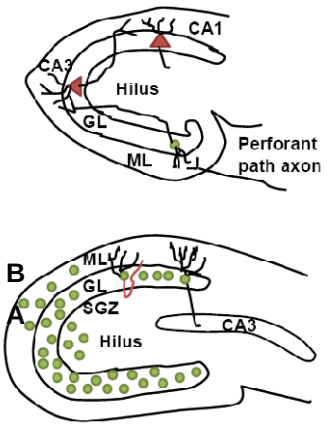

Figure 1.2: Schematic outline of the hippocampal circuit *(A)* and an epileptic dentate gyrus showing granule cells that migrated towards the ML and hilus (GCD) and also aberrantly projects to the inner ML (MFS) *(B). SGZ,* subgranular zone; *GL,* granular layer.

During the last decades, several studies on epileptic human temporal lobe tissues have shown that these pathophysiological changes are not similar in all patients. Some patients, usually those with refractory TLE develop hippocampal sclerosis, which is characterized by the loss of dentate hilar neurons and CA1 and CA3 pyramidal neurons[16]. Yet, the molecular mechanisms that underlie the development of hippocampal sclerosis are still unclear. Some studies suggest that differential gene expression in the hippocampus may play a role[3, 7].

Another pathophysiological feature of the epileptic hippocampus is granule cell loss and more often granule cell dispersion (GCD) to the hilus and inner molecular layer, which is present in 40-50% of the patients[17-19]. Studies suggest that these aberrantly migrated granule cells are hyperexcitable and integrate

abnormally[20]. Moreover, the epileptic hippocampus also shows mossy fiber sprouting (MFS), which according to anatomical and electrophysiological evidence, seems to increase recurrent excitatory circuits as the axons of dentate granule cells project abnormally to the supragranular inner molecular layer of the dentate gyrus. Alternatively, mossy fiber reorganization may also enhance recurrent inhibition within the dentate gyrus[3, 17, 21] (see figure 1.2).

Neurons as well as astrocytes are affected by the initial insult and recurrent seizures. Following stressful stimuli, astrocytes upregulate the glial fibrillary acidic protein (GFAP) and its cell body and processes show hypertrophy. This process is called reactive gliosis and is present in several acute and chronic CNS diseases. Further, the organization of astrocytes in non-overlapping spatial domains can be lost during recurrent seizures, which could worsen the pathophysiology[22].These pathophysiological changes in the hippocampus imply that the dentate granule cells play a central role in the pathology of TLE[17]. Studies on animal models suggest that these progressive changes initiate directly after acute seizures[16, 23]. However, it is not clear whether they are involved in the development of chronic seizures. The concept referring to the neurobiological changes that occur after the initial injury and are involved in the development of chronic seizures, are referred to as epileptogenesis[3]. Some believe that epileptogenesis is the result of synaptic reorganization that occurs after cell death[24]. Nevertheless, the mechanisms underlying this concept remain obscure.

One of the first theories on the mechanism underlying epileptogenesis is the dormant basket cell theory, which gives a possible explanation to dentate hyperexcitability. This hypothesis postulates that the loss of mossy cells leads to the excitatory denervation of interneurons, which results in the inhibition of granule cells[25]. Yet, in the past decades several other hypotheses have been postulated (for further details see Buckmaster et al., 2002; Aroniadou-Anderjaska et al., 2008). These hypotheses will not be discussed in further detail as they fall beyond the scope of this thesis. More recently, neurogenesis was proposed to be potentially involved in epileptogenesis. This theory has been based upon the interesting findings that neurogenesis still occurs in the adult hippocampus and that it is affected by seizure activity in animal models. However, it remains to be determined whether neurogenesis does play a role in epileptogenesis. Indeed it is of great interest to clarify the mechanisms underlying epileptogenesis as this will result in vast improvement of current treatment for TLE.

Therefore, the aim of this thesis is to determine whether there is an association between neurogenesis and epileptogenesis. To this end, the effect of acute and chronic seizure activity on neurogenesis will be reviewed and it will be discussed whether neurogenesis could have a causal relation to epileptogenesis. The next chapter will begin with a brief overview of the cellular and molecular mechanisms underlying neurogenesis. In the second section, the effect of acute and chronic seizure activity on cell proliferation will be reviewed. Thereafter, it will be discussed whether neurogenesis could be involved in epileptogenesis and to what extent the developing and aged brain and the severity of SE may influence neurogenesis. The final section will discuss whether the results shown in rodent models can be significant for human TLE patients and how this affects the current theories. Finally, it will be discussed whether an association between neurogenesis and epileptogenesis is probable in light of the current knowledge.

Chapter 2

Neurogenesis and its Relevance to Epileptogenesis in Temporal Lobe Epilepsy

2.1 The Molecular and Cellular Mechanism of Neurogenesis in the Temporal Lobe

Until recently, it was generally believed that neurogenesis took place only during brain development. In recent years, studies on rat and mice models identified proliferating cells in the hippocampal dentate gyrus and olfactory bulb, providing compelling evidence that neurogenesis occurs throughout the mammalian adult life. These proliferating cells were identified by a marker specific for dividing cells *viz.* the thymidine analog bromodeoxyuridine BrdU, which is integrated into DNA during the S-phase of the cell-cycle[14, 26, 27]. It has been shown in the rodent dentate gyrus that around 6% of the total cell number is integrated into the hippocampus every month[26]. Interestingly, a study using long-term genetic labelling of neural stem cells in the dentate gyrus suggests that the new born neurons contributes to increase the number of granule cells during adulthood[27a]. These new neurons arise from progenitor cells, which give rise to either neurons or glial cells and a daughter progenitor cell. The newborn neurons can be identified by using the neuronal nuclear specific protein (NeuN) as a marker for young mature neurons. The progenitor cells are located in the subgranular zone (SGZ) of the dentate gyrus and migrate into the granule layer (GL) where they develop dendrites towards the molecular layer (ML) and axons that contact the pyramidal cells in the CA3 layer[27]. There is also evidence suggesting that astrocytes can be primary precursor cells for neurons. These cells divide once, forming D1 cells that also divide generating D2 cells, which in turn extend processes towards the GL forming D3 cells, which will become mature granule cells[26].

Several factors are known to regulate neurogenesis during adulthood. For instance, the number and proliferation rate of the progenitor cells determines the extent of neurons that arise in the dentate gyrus. Furthermore, the release of mitogenic factors due to cell death and the increased levels of proliferation factors (*e.g.* fibroblast growth factor-2 (FGF-2), insulin growth factor-1 (IGF-1), neuropeptide Y, vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF)) stimulate neurogenesis. Furthermore, BDNF also promotes cell survival and maturation of precursor cells[28, 29]. Also a high serotonin level, low glucocorticoids level, enhanced neuronal activity and the proximity to vascular niche promotes proliferation, cell survival and differentiation[30]. In contrast, stress hormones such as, adrenal steroids, were shown to suppress neurogenesis[31, 32].

The new neurons formed in the SGZ migrate towards the GL. However, the molecular mechanism that underlies this migration is still unclear. Some studies showed that the radial glial

fibers extend from the subgranular zone (SGZ) and traverse the GL perpendicularly, providing a scaffold for the migrating neurons. Intriguingly, the mouse mutant *reeler,* which lacks the protein reelin, shows an inverted cortex as neurons migrate aberrantly to the superficial plate[20], suggesting that this protein may function as a stop signal for migrating neurons. Reelin is an extracellular protein synthesized and secreted by Cajal-Retzius cells, which are located in the marginal zone of the cortex, and by inhibitory GABAergic interneurons in the hilus and ML of the dentate gyrus. However, it is not yet clear how reelin regulates neuronal migration[20, 33].

Several studies have shown that the local environment provided by astrocytes is important to promote neurogenesis in the dentate gyrus. For instance, it was demonstrated that neuronal precursor cells from non-neurogenic regions differentiate into neurons when transplanted into the dentate gyrus, suggesting that astrocytes from non-neurogenic regions such as the spinal cord are incapable of promoting neurogenesis of neuronal precursor cells[34, 35]. Hence, astrocytes secrete soluble and membrane-associated factors, such as Wnt-3, that are essential for an environment that supports neurogenesis. Besides regulating cell proliferation, these factors may also be involved in neuronal fate determination and promoting neuronal survival and maturation[26, 34, 36]. There is emerging evidence that astrocytes form non-overlapping spatial domains in the dentate gyrus, which may be yet another mechanism to restrain neurons from migrating aberrantly towards the hilus and ML[22].

After the young neurons have migrated to the GL they become functionally integrated to the hippocampal network. This integration follows a stereotypical pattern as the young neurons first receive GABAergic synaptic input followed by glutamatergic synaptic innervation[37]. Thus, new neurons may sense neuronal activities through local GABA levels, suggesting a role for GABA in regulating functional integration.

The majority of neurons can survive for prolonged periods and develop into mature neurons showing the morphological structure and functional synaptic properties of existing granule cells[30]. Moreover, they have the capacity to receive excitatory as well as inhibitory synaptic inputs and send synaptic outputs. Therefore, newborn mature granule cells present the synaptic responsiveness and other electrophysiological properties of granule cells, although synapse formation and response are decreased in comparison to existing granule cells[36, 27].

In conclusion, the cellular and molecular mechanisms promoting neurogenesis are becoming clear. This knowledge will prove invaluable in understanding neurological developmental disorders, as evidence shows that primary precursors do not develop properly without the required intrinsic and extrinsic factors.

2.2 The Seizure-Effect on Neurogenesis in Temporal Lobe Epilepsy

The intriguing finding that some areas in the adult mammalian brain still retain their capacity to support neurogenesis, has led some to see it as a possible mechanism underlying the development of several neurological disorders and as a potential treatment for these diseases. As mentioned previously (see section 2.1), several factors are known to promote neurogenesis. One of these factors is neuronal activity, which can be enhanced by exercise and enriched environment[35]. This notion has led some to propose that seizure activity may also increase neurogenesis in the dentate gyrus as it enhances neuronal activity as well. Therefore, in the past years, several studies have addressed this issue, yielding contradictory results. While some showed supporting evidence, others did not. These contradicting findings may result from studies being

conducted in different animal models, such as perforant pathway activation and kindling[38]. However, most studies have been performed in pilocarpine or kainic acid induced seizure models as they most resemble the behavioural and pathophysiological aspects of TLE in humans.

The effect of acute and chronic seizures on neurogenesis

Several studies treated male adult Sprague-Dawley rats intraperitoneally (IP) with pilocarpine, showing that neurogenesis increases during acute epilepsy in this rat model [17, 39, 40] (reviewed by Scharfman H.E., 2007 and Parent J.M., 2007). In contrast, when adult male rats or mice are injected with kainic acid either intracerebroventricularly (ICV) or IP, some results confirm previous findings while others show evidence suggesting that neurogenesis actually decreases in the injected area and increases in the contralateral hippocampus [19, 23, 28, 41, 42]. The difference observed between the studies may be caused by the fact that SE models have different characteristics, *e.g.* the rat or mouse strain, the chemoconvulsant used and the method of injection, which have been shown to determine pathophysiology and the extent of hippocampal damage.

The IP KA model induces acute seizures that result in bilateral loss of hilar neurons, CA1 and CA3 pyramidal cells. This phase is followed by a latent period during which several epileptogenic changes take place leading to chronic epilepsy[28, 41, 43]. In this model, an increase in neurogenesis was observed during the acute phase (see figure 2.1). This finding is also supported by studies by Gray and Hattiangady that used a KA model where adult male Wistar mice and Sprague-Dawley rats received a unilateral ICV injection of kainic acid in the left ventricle. This resulted in an initial status epilepticus (SE), followed by a latent period, after which the mice developed ipsilateral Spontaneous Recurrent Seizures (SRS)[28, 42]. In this KA model, the contralateral side also showed enhanced neurogenesis because both hippocampi undergo epileptogenic changes that induce hyperexcitability in the dentate gyrus. Intriguingly, more recent studies, which also used an ICV KA model, found that neurogenesis decreased in the injected dentate gyrus and increased in the contralateral side[19, 23, 44]. These findings, which seem contradictory, may be explained by the observation that this ICV KA model causes extensive damage in the CA1 and CA3 areas of the injured side as the injection is given directly in the left CA1 area. In contrast, the contralateral dentate gyrus does not show any evident sign of damage[19, 23, 44]. Therefore, it may be possible that due to the extensive physical injury caused by the injection itself in the CA1 area, the ipsilateral dentate gyrus is no longer capable to maintain an environment that supports neurogenesis. Interestingly, it has been demonstrated that neurogenesis increases temporarily, returning to baseline after 3 months[43]. Moreover, it has been shown in the IP and ICV KA models, that neurogenesis substantially declines during the chronic phase[28] (see figure 2.2). However, the IP KA model showed a significant higher decrease in cell proliferation, probably due to the higher frequency of spontaneous recurrent motor seizures (SRMS) in this model. These results imply that the higher SRMS frequency during the chronic phase causes more damage to cells in the dentate gyrus.

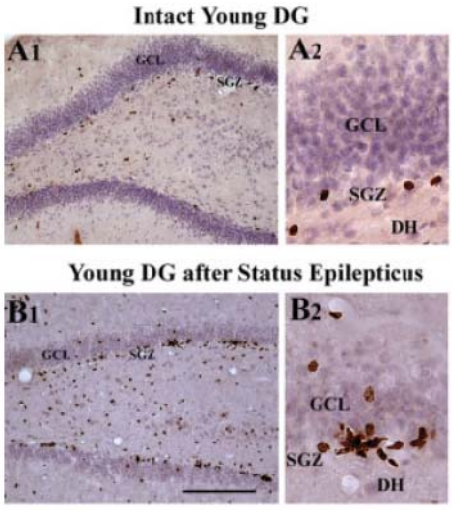

Figure 2.1: This figure shows the BrdU labeled cells in the dentate gyrus from rats without KA injection (A) and after SE (B). A2 and B2 are magnified views of A1 and B1. (From Rao MS., et al., 2008.)

There are striking similarities between the studies that used an ICV KA model that shows extensive damage in the ipsilateral dentate gyrus, and the pattern of KA-induced lesions during the chronic phase[28]. This suggests that the effect of seizures on neurogenesis was estimated in a model for chronic seizures and not in a model representative for the acute phase.

Factors that influence the neurogenic potential of the hippocampal environment

Besides the vast damage caused by the ICV KA injection other factors, such as the genetic background, may also influence the neurogenic potential. Studies comparing the effect of seizures between mice and rat strains, demonstrated that some strains are more sensitive to SE induction and develop more extensive damage than others[8, 16]. However, it does not seem that there is a correlation between differential gene expression and neurogenesis, at least not for the mice or rat strains so far investigated.

The results are not only determined by which KA model is used, but also by the time-point when BrdU is administered and by the other markers used to identify cell proliferation. As mentioned above, proliferative cells are identified with BrdU, which detects cells in the S-phase of the cell-cycle or their post-mitotic daughter cells[14]. After administration, rapid DNA labelling takes place for about 30-40 minutes and incorporation at lower levels continues for 4-8 hours, after which the level of the marker is too low to be sufficiently incorporated for detection[45]. Hence, it is essential to take the clearance time into account, as the number of actual proliferating cells may be higher than the amount of $BrdU^+$ cells that were counted. Further, the time-point of BrdU injection relative to the KA administration determines the effect that will be observed on the proliferation of precursor cells following seizure.

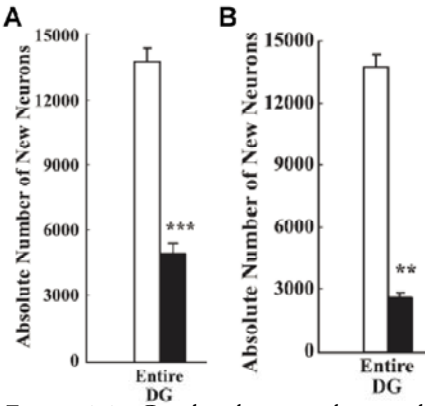

Figure 2.2: Graphs showing decreased neurogenesis (black bar) in the dentate gyrus 5 months after ICV KA (A) and IP KA (B). Intact hippocampus from age-matched control (White bar)
*** = p< 0.001 ** = p<0.01 (From Hattiangady B., et al., 2004.)

Thus, by administrating BrdU before KA treatment, the seizure effect on cells that were already proliferating before KA-induced seizure can be assessed and by injecting BrdU after or simultaneously with KA, the effect of seizure on triggering and supporting cell proliferation can be determined. Another factor that one should keep in mind is that BrdU may be taken up into the nuclei of injured or mature neurons[17]. Thus, sometimes an additional measure for neurogenesis is used, *i.e.* the microtubule-associated phosphoprotein doublecortin (DCX), which is an excellent marker as it is expressed after 3 hours in neuronally committed newborn cells[41].

The molecular mechanisms underlying aberrant cell proliferation following seizures

As mentioned before (see section 2.1), seizures enhance neuronal activity, which may stimulate neurogenesis by direct synaptic activation of some mitotically active precursor cells that seem to receive synaptic contacts[17, 35]. Furthermore, a study showed that pharmacological inhibition of NMDA receptor-mediated excitatory input, positively regulates the proliferative rate of precursor cells in the dentate gyrus[32, 35]. The NMDA receptors on granule cells are thought to suppress proliferation by increasing the release of glutamate under regulation of glucocorticoids. However, it is still unclear how exactly this stress hormone modulates the NMDA receptor channel[46]. Alternatively, enhanced neuronal activity might stimulate cell proliferation by modulating the expression of growth factors. Some studies have shown that the expression of several genes

changes since the initial insult, implying that they play a pivotal role in the pathophysiology and epileptogenesis of TLE [8, 9, 35].

Evidence shows that the growth factors IGF-1, BDNF and Wnt3 are upregulated in the human epileptic dentate gyrus[30, 41, 47]. Besides, it has been demonstrated *in vivo* that these proliferation factors are important to ensure an environment that supports cell proliferation. For instance, deletion and overexpression of the *igf-1* gene, respectively reduces and increases neurogenesis[48]. However, it is still obscure how IGF-1 upregulates cell proliferation. In addition, BDNF was shown to increase neurogenesis when administrated ICV, while heterozygote BDNF knock-out mice show impaired cell proliferation. These results suggest that BDNF is involved in regulating neurogenesis. This protein is expressed by granule cells and is a member of the neurotrophin family of proteins that are known to interact with the receptors p75 and TrkB, which have been implicated in regulating cell proliferation and survival[49, 50]. BDNF may regulate cell proliferation through the receptor TrkB that activates among other signalling pathways, the mitogen-activated protein kinase (MAPK), which induces the expression of several immediate early genes (IEG) including genes that are involved in cell proliferation, such as *G1 cyclins*[51]. BDNF may also indirectly promote neurogenesis by modulating the expression of the neuropeptide Y, which is known to enhance cell proliferation[29].

Some of these neurotrophic factors, such as Wnt3, are expressed by astrocytes, which secrete several other factors that stimulate neurogenesis. It has been shown that inhibition of Wnt3 signalling results in a significant decrease in neurogenesis[47]. The effect of Wnt3 on cell proliferation may be mediated through its direct interaction with Wnt and Wnt/β-catenin receptors on the surface of precursor cells thereby activating the Wnt pathway, which stabilizes β-catenin by suppressing its inhibition. Stabilised β-catenin will then accumulate in the cytoplasm and enter the nucleus, thereby coactivating the transcription factor LEF-1/TCF. This transcription factor activates several genes, among which the *c-myc* that encodes for the protein c-Myc, a strong stimulator of cell growth and proliferation[52].

Besides producing several factors that are essential for neurogenesis, astrocytes also provide structural, metabolic and tropic support for proliferating cells. In addition, accumulating evidence suggests that these cells are crucial for providing an environment that supports neurogenesis (see section 2.1). Astrocytes comprise almost half of the cells in the dentate gyrus and are in direct contact with proliferating cells *in vivo* and in proximity to vasculature[34], which has been implicated in promoting neurogenesis. Several studies have shown that endothelial cells are recruited within the subventricular zone (SVZ), possibly due to enhanced metabolic demand. This may be mediated by astrocytes, as studies have shown that they release vasodilating substances in response to increased glutamate levels. However, the vascular niche seems to play a more active role in promoting neurogenesis, as the endothelial cells express BDNF. However, how exactly this vascular niche provides a supportive environment for neurogenesis is still obscure. There may be a common factor or mechanism that leads to increased vascularisation and neurogenesis. For instance, the growth factor FGF-2 that is secreted by astrocytes does not only promote cell proliferation, but acts also as an angiogenic factor[53].

Alternatively, FGF-2 may promote neurogenesis by upregulating the expression of another mitogen, the VEGF, and the endothelial VEGF-receptor Flk-1. This receptor was found to be expressed in progenitor cells of the mouse retina and may also be present in hippocampal precursor cells. Further, several studies have shown that VEGF acts upstream of BDNF, implying that these two proteins may be involved in the same pathway regulating neurogenesis[50].The

important role of VEGF and its receptor on cell proliferation was further supported by a study showing that inhibition of the Flk-1 receptor with the tyrosine kinase inhibitor SU1598, suppressed VEGF stimulated neurogenesis [10, 54].

Both the acute and chronic phase induced in the ICV and IP KA seizure models, present apoptotic cells. Intriguingly, neurogenesis decreases during chronic seizures even though significant cell death occurs[28]. This may be due to the depletion of precursor cells as was demonstrated in a study that showed a decline in cells positive for Vimentin, a marker for radial glial cells or putative stem cells[55]. In contrast, TLE patients show an increased number of cells positive for Vimentin and Musashi-1 in the SGZ. This is interesting as Musashi-1, a RNA binding protein, is a marker specific for immature neural cells. This protein is also involved in the Notch signalling pathway, which instructs precursor cells to remain either undifferentiated, or proliferate and differentiate into glia cells[1]. Hence it seems that the decline in neurogenesis during the chronic phase in TLE patients is related to an impairment in cell fate determination and not necessarily to decreased cell proliferation or number of progenitor cells.

Another possible cause for the decline in neurogenesis during chronic seizures may be that the dentate gyrus environment no longer supports neurogenesis, which is supported by the finding that the growth factors BDNF, FGF-2 and IGF-1 are reduced in chronic seizures. Although inflammation suppresses neurogenesis, a previous report showed that inflammation was increased when neurogenesis was enhanced and was declined when neurogenesis decreased, implying that chronic inflammation is not likely involved in reducing cell proliferation[28]. Nevertheless, these are still preliminary findings and more studies need to be done to establish which mechanisms underlie the pronounced decline in neurogenesis.

Thus far, growing evidence suggests that hippocampal neurogenesis is modulated during acute and chronic seizures in KA and pilocarpine-induced seizure models. As discussed above, the results can be influenced by the extent of hippocampal damage and by the time-point of BrdU injection. Therefore, the method of SE-induction and when BrdU will be administered should be carefully chosen.

2.3 Neurogenesis and its Role in Temporal Lobe Epileptogenesis

It is becoming widely accepted that, at least in adult rodent models, seizure activity affects hippocampal cell proliferation. A substantial fraction of newly born cells were shown to migrate into the granule cell layer and develop morphological and electrophysiological features of mature dentate granule cells[17, 27]. Even so, some studies suggest that newborn cells mature more slowly after induced seizures[44, 56]. During the last years, studies have analysed whether aberrant cell proliferation contributes to the development of *e.g.* granule cell dispersion and synaptic reorganization (see section 2.1). Therefore, it has been postulated that an aberrant maturation of newborn neurons may play a role in the development of chronic epileptic seizures.

Cell fate determination

Neuronal differentiation is determined by the hippocampal environment. The epileptic hippocampus presents several changes in the environment, which may result in impaired cell fate determination, since only 4% of the new cells differentiate into neurons compared to 80% in the age-matched intact hippocampus[1]. It has been shown that the one-to-one relationship between newborn neurons and astrocytes is disrupted, which may affect differentiation as cell-cell contact between astrocytes and progenitor cells in the SGZ seems to be important for cell differentiation[26, 57]. A recent study assessed the differentiation of progenitor cells in a unilateral ICV KA-induced SE model by using a marker for differentiating neurons, *viz.* DCX and NeuN. The results showed that the amount of $BrdU^+/DCX^+$ cells in both the ipsilateral and contralateral hippocampi is lower than in the saline injected control, implying that ICV KA-induced SE impairs cell differentiation (see figure 2.3). Further, it also demonstrated that most cells prelabeled with BrdU differentiated into neuronal cells, like in the control group, while 70-90% of the cells labelled with BrdU after KA treatment were $GFAP^+$ (glial fibrillary acid protein), which is a marker for astrocytes and glial cells[44]. These results illustrate the importance of the time-point of BrdU injection relative to SE induction and demonstrate that at least in these experimental conditions, SE does not affect the differentiation of neuronally committed cells, while cells proliferating after SE induction show aberrant cell fate determination. However, the SE model used in these studies presented substantial damage in the CA1 and CA3 areas and extensive loss of hilar neurons as kainic acid was injected directly in the CA1 area. Therefore, one cannot conclude with certainty whether impairment in cell differentiation is due to the induction of SE or to the extensive damage, which induces significant changes in the environment that may diminish cell fate determination. The extracellular changes seen in this SE model are not only caused by the chemoconvulsant but also by the extensive cell death due to the inflicted cellular damage. Thus, this model may not correlate with other less detrimental models and it is possibly not representative of the actual biological changes occurring in TLE patients.

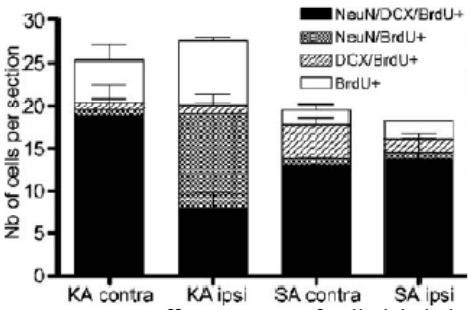

Figure 2.3: Differentiation of cells labeled with BrdU before ICV KA treatment. Both KA treated hippocampi show less DCX^+ cells in contrast to the age-matched control, suggesting a disruption in cell differentiation.
(From Ledergerber D., et al., 2006.)

As said above, this impaired differentiation is possibly due to the change in expression of several factors, such as Musashi-1 (see section 2.2). Interestingly, a study demonstrated that cells in the dentate gyrus co-express Musashi-1 and $p27^{Kip1}$, a protein that indicates the end of division and the onset of differentiation of neuronal progenitor cells. This suggests that the precursor cells proliferate, yet remain in an undifferentiated state[1, 55]. Another protein that is downregulated in SE models is DCX. This protein may regulate differentiation as it is downstream of reelin, which has been implicated in regulating cell fate determination[33]. Further, following reactive gliosis the expression of the extracellular matrix glycoprotein Secreted Protein, Acidic and Rich in Cysteine (SPARC) is upregulated. This suggests that cell differentiation is impaired as this protein negatively regulates several growth factors involved in cell fate determination[56a, 57].

Thus, several lines of evidence suggest that newborn neurons have impaired cell fate determination. The morphological and functional features of newborn cells were analysed in

Sprague-Dawley female and male rats by injecting BrdU intraperitoneally and directly infusing Semliki Forest Virus [pSFV(*pd)*] vectors expressing the green fluorescent protein (GFP) in both hippocampi[57a]. Interestingly, only 14% of the $BrdU^+/GFP^+$ newborn neurons showed morphological features of GABAergic cells, which are important to ensure that the newborn neurons mature and integrate properly[41]. Therefore, an impaired cell differentiation may contribute to epileptogenesis. However, additional studies are required to determine to what extent impaired cell differentiation would contribute to epileptogenesis.

Aberrant migration of granule cells

As discussed in section 1.2, one of the pathophysiological features of TLE is granule cell dispersion to the hilus and inner molecular layer of the dentate gyrus. These cells are hyperexcitable, as they show abnormal burst firing that occurs synchronously with CA3 pyramidal cells[38]. Hence, these cells may be implicated in the epileptogenic process of TLE.

As mentioned previously (see section 2.1), astrocytes seem to form non-overlapping spatial domains in the dentate gyrus, forming a dense network of basal processes that isolate the progenitor cells from the hilus. This organization may prevent new born neurons from migrating aberrantly towards the hilus and ML, by functioning not only as a scaffold for migrating neurons, but also by releasing neurotrophic factors. Interestingly, a recent study investigated the astrocytic domain organization of cortical astrocytes in the IP kainate SE mice model by using diolistic labelling of brain slices, demonstrating that reactive gliosis following seizures may disrupt the astrocytic domains[22, 26]. Although this study focused on cortical astrocytes, there is also evidence that hippocampal astrocytes form similar domains. Thus, as mentioned previously (see section 2.1), this structural organization may prevent hippocampal newborn neurons from migrating aberrantly towards the hilus and molecular layer[22]. The fraction of newborn neurons that migrate aberrantly towards the hilus and ML *i.e.* ectopic granular cells (EGC) has been measured in the intact and epileptic dentate gyrus in adult rats. This study demonstrated that the amount of EGC increased substantially following IP KA induced SE, increasing from about 3,5% to 34%. These results suggest that new granule cells may have a significant contribution to the formation of GCD (see figure 2.5) [41, 58, 59].

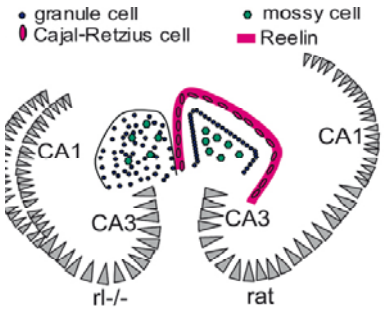

Figure 2.4: A schematic overview of the hippocampus of a wild-type rat and a *reeler* mutant showing the effect of reelin deficiency on neuronal lamination. (From Zhao S., et al., 2004.)

The molecular mechanisms underlying granule cell dispersion are becoming clear. A recent *in vitro* study suggests that reelin may not only function as a stop signal for migrating neurons, but also as a factor regulating the detachment of these cells from the radial glial scaffold[33, 20]. Reelin may interact with neurons and radial glia as it has been shown that they express the reelin receptors apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR), and the downstream protein Disabled1 (Dab1). Hence, reelin may bind to its receptors on neurons and radial glia, thereby inducing the phosphorylation and degradation of Dab1, which induces new born neurons to detach from the radial glial fiber and migrate towards the GL. Another downstream protein DCX, is known to be involved in cell motility and outgrowth and may be a target protein in yet a different pathway through which reelin regulates migration[44]. Further, reelin may also directly act on the radial glial scaffold as radial glial processes

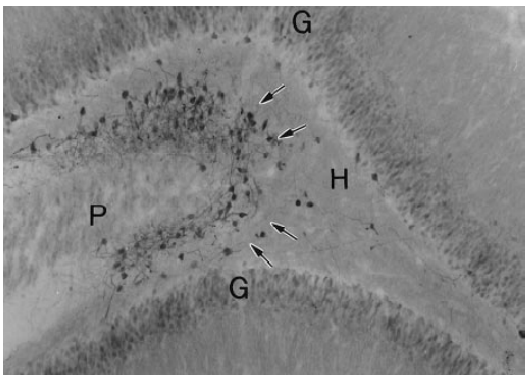

Figure 2.5: Aberrantly migrated newborn neurons positive for Calcium-binding protein calbindin D_{28K} (CaBP) at the hilar/CA3 border (arrows). This marker identifies granule cells and a few GABA neurons.
H, hilus; *G*, granule cell layer; *P*, pyramidal cells. (From Scharfman HE., et al., 2000.)

are abnormal in the *reeler* and *scrambler* mice, which are mutants that lack the reelin receptors and Dab1, respectively (see figure 2.5)[33, 20].

Interestingly, two previous studies in rats and mice assessed whether neurogenesis contributes to the formation of GCD in an ICV KA-induced seizure model, showing that the injected hippocampus had decreased neurogenesis accompanied with substantial GCD, while the contralateral side presented enhanced neurogenesis without GCD[19, 23]. These results led some to suggest that neurogenesis is an adaptive mechanism protecting the hippocampus from epileptogenesis and that GCD is associated with the absence of neurogenesis. It has also been proposed that GCD possibly results from the decrease in reelin synthesis, which leads to the displacement of mature neurons but not of newborn neurons[19, 23]. However, it can be argued that the seizure model used in these studies is not suitable to determine a causal relation between neurogenesis and GCD, because the injection itself will induce physical damage in the hippocampus. As discussed above (see section 2.2), the extensive damage caused by the injection itself may impair the ability of the dentate gyrus environment to support neurogenesis. This additional damage to the injected hippocampus is not taken into account in the interpretation of the data. Therefore, the conclusions advanced in the studies above may not be entirely justified. Based on the arguments above, it can be concluded that these studies clearly demonstrate that neurogenesis is not an essential process for the development of GCD and therefore do not preclude that neurogenesis does not play a role in GCD.

Furthermore, the studies mentioned above have demonstrated that the ipsilateral hippocampus has a significant reduction in the number of reelin synthesizing cells, in contrast to the contralateral side, which shows no alteration in the amount of reelin production[23]. It has also been shown that ICV KA-induced seizure upregulates BDNF, which is known to negatively regulate reelin expression in the dentate gyrus. This seems to be an important mechanism, as infusion of BDNF with a cannula directly into dorsal hilus of the dentate gyrus leads to ECGs even in an undamaged hippocampus[29]. Considering that reelin deficiency is an important factor in the development of GCD (see section 2.3 above), it can be proposed that GCD is due to the absence of reelin, rather than to the lack of neurogenesis (as proposed in refs. [19, 23]).

In conclusion, although several lines of evidence suggest that neurogenesis may be involved in the formation of GCD, it has yet to be proven indisputably whether or not newborn neurons play a role in this process. In order for this to be determined it is important to establish whether the newborn neurons migrate aberrantly towards the hilus/CA3 border early during epileptogenesis and if so, to what extent they may contribute to the formation of GCD. Alternatively, newborn neurons may contribute significantly to GCD during the chronic phase of epilepsy, in which case they will be involved in the maintenance of the epileptogenic process.

Synaptic organization of newly born granule cells

Mossy fibers sprout to the inner molecular layer forming connections with granule cells and interneurons. Abnormal integration of ECGs and GCs in the GL or hilus possibly contributes to hyperexcitability, which may increase seizure activity. This in turn, could lead to further loss of reelin resulting in more aberrant migration[20]. Axonal remodelling and synaptic reorganization of mossy fiber axons increases with repeated seizures and alters connectivity and circuits in the dentate gyrus, which may contribute to the recurrent excitation underlying epileptogenesis (see section 1.2). It is very likely that seizure activity changes factors that regulate the destination of mossy fibers [60, 61]. Furthermore, there is some evidence suggesting that neurogenesis could contribute to the formation of MFS[17]. For instance, it has been demonstrated that $BrdU^+$ cells in the GL send neurofilament protein (NF-M) stained axons to the ML, implying that newly born neurons are involved in the development of MFS (see figure 2.6). Furthermore, it has been shown that 3 weeks old granule cells are hyperexcitable and have increased Ca^{2+} conductance. This may contribute to epileptogenesis as active NMDA receptors activate the Ca^{2+} transduction pathway, which cause the development of spontaneous recurrent discharges [5, 27].

In contrast, it has also been shown that ablation of neurogenesis by irradiation does not prevent or significantly reduce MFS, suggesting that essentially mature granule cells contribute to mossy fiber remodelling. On the other hand, some have proposed that the developing granule cells may inhibit new axon collateral outgrowth from mature granule cells. Furthermore, extensive loss of CA3 pyramidal neurons and some loss of hilar neurons have been associated to robust MFS, suggesting that EGCs may develop afferent input due to their close proximity to axons that have lost their hilar target cell[16, 21, 59]. Other findings suggest that newborn granule cells have basal dendrites that may be a target for recurrent mossy fiber synapses[57]. However, other studies demonstrate that ECGs in the hilus originating from newborn neurons form functional circuits and maintain enhanced plasticity even after they mature[59].

As mentioned before, the integration of new neurons in the hippocampus follows a similar pattern as during neuronal development in other brain areas, *i.e.* the young neurons first receive a depolarizing GABAergic synaptic input followed by glutamatergic synaptic innervation (see section 2.1)[37]. Thus, GABA likely regulates functional integration of newborn neurons, suggesting that during the maturation process new neurons are initially hyperexcitable and have a high $[Cl^-]_i$, and as a result GABA has a depolarizing effect, which is necessary for normal maturation[37]. During this period the neurons become sensitive to glutamate activation and GABA inhibition, possibly because of an increase in threshold potentials. This process could ensure that newly born neurons mature properly. Therefore, because during the chronic phase a substantial fraction of GABA interneurons is lost and neurogenesis decreases, the dentate gyrus will contain much less GABA interneurons. Thus, the new neurons will not mature and integrate properly in the dentate gyrus[28, 41, 53].

A recent study has shown that chronic seizures decrease after inhibiting seizure-induced

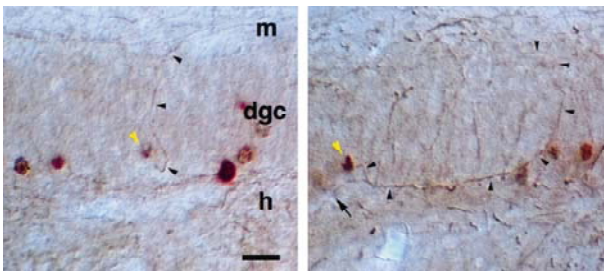

Figure 2.6: Double-labeled immunohistochemistry with BrdU and NF-M, which is a marker for the protein neurofilament, demonstrates that newborn cells send aberrant axons projections to the ML. Yellow arrowheads indicate $BrdU^+$ cells and black arrows delineate the trajectory of NF-M stained axons. Scale bar 25 µm. *h*, hilus; *dgc*, dentate granule cell layer; *m*, molecular layer. (From Parent JM., et al., 1997.)

neurogenesis with the mitotic inhibitor cytosine-β-D-arabinofuranoside[41]. In contrast, other studies showed that the absence of neurogenesis did not prevent the development of chronic seizures. Furthermore, it has been shown that seizure-induced changes in the hippocampal environment, such as enhanced BDNF expression, increases the synapse formation and synaptic transmission of newborn granule cells[21, 36, 44, 62]. Thus so far, evidence suggests that increased neurogenesis is not essential for the development of mossy fiber sprouting.

As discussed in the previous section SE- induced models present different injury patterns, which are likely to confound the results if not taken into consideration. For instance, the ICV KA model shows GCD and extensive neuronal damage in the CA3 area, while the IP KA shows less damage, GCD and MFS[23, 28, 58, 63]. Because of these differences in the models, current studies have shown contradictory findings supporting either that aberrant cell proliferation has a substantial contribution to epileptogenesis or that it plays no role in the development of chronic seizures. It seems likely that several factors underlie epileptogenesis, contributing at different degrees. At present, it seems that aberrant neurogenesis is not essential to epileptogenesis. It has yet to be established to what extent newborn granule cells contribute to GCD and MFS. Future studies need to determine the significance of impaired cell fate determination to epileptogenesis.

2.4 The Effect of the Developing and Aged Brain and the Status Epilepticus Severity on Neurogenesis

Thus far, little is known about the biological mechanisms that influence neurogenesis throughout life. Although, it has been established that acute seizures increase cell proliferation in the dentate gyrus of adult rodent models, much less is known about the age-dependent mechanisms affecting neurogenesis. It is well known that experiencing febrile seizures early in childhood significantly increases the risk in developing temporal lobe epilepsy later in life. Therefore, it is of great interest to understand how an early seizure episode can affect brain development and contribute to epileptogenesis. Further, the chance to develop chronic epilepsy also increases with age, yet several studies have determined that seizure-induced proliferation decreases in the aged brain[41]. But, how these different responses to seizure activity are modulated is not yet clear.

The human and rat brain develops for the most part after 16 weeks of gestation and 15 days postfertilization, respectively. However, the hippocampus and cerebellum develop later, completing formation after 34 weeks of gestation in humans and the first two postnatal weeks in rats[64]. During the first week, several cells are proliferating and are dispersed throughout the dentate gyrus. By P13, the majority of the $BrdU^+$ cells have migrated to the GL and by P20 show morphological features of mature granule cells. Interestingly, neurogenesis substantially increases between P6 and P9 and gradually declines to baseline level by P30[65]. Thus, the immature hippocampus initially promotes neurogenesis for a brief period, which is followed by molecular and cellular changes that reduce cell proliferation. Intriguingly, it seems to make a difference whether seizures are induced in the first week or later, as it has been reported that one week old rat pups show decreased neurogenesis, whereas two weeks to four weeks old pups show an increased neurogenesis (reviewed by Porter BE., 2008)[65-69]. One could say that this implies that seizure activity induces changes that impair neurogenesis during the first week. Interestingly, seizures induced in rat pups barely damages the dentate gyrus, in contrast to seizure models in

adult rats, which develop extensive damage (see section 2.2). Therefore, it seems that for some reason the changes in the young pup are more intrinsic.

The mechanisms that mediate seizure-induced changes on neurogenesis in the young rat pup are still obscure. A study in the IP KA induced seizure model in P6-P30 old rat pups has shown that the level of glucocortisteroids is inversely correlated to the number of $BrdU^+$ cells[65]. Considering that glucocortisteroids inhibits cell proliferation (see section 2.2), it seems that the seizure mediated increase in glucocortisteroids may be responsible for the decline in neurogenesis during the first week. Interestingly, the same study also showed that the expression of the growth factors BDNF, FGF and NGF are not increased in pups, in contrast to adult rats (see section 2.2). Furthermore, there is increasing evidence suggesting that cell death is not essential for aberrant cell proliferation in two weeks old rat pups, as the pilocarpine induced SE-model did not show any significant cell loss[68, 69]. In contrast to the results in other seizure models, febrile seizures (FS) in pups from P10-P11 days old did not cause any change in the hippocampal neurogenesis, but instead led to long-term axonal reorganization[67]. This change was not accompanied to acute cell death, as FS only causes some temporary hippocampal injury. Thus, these findings illustrate the difficulty to study seizure induced changes in the young pub brain.

Intriguingly, it has been demonstrated that in the young rat pup, more seizures are necessary to change cell proliferation significantly[65, 67, 69]. This may be because the cellular organization of the dentate gyrus is not complete until around the end of the second week. This suggests the interesting possibility that the precursor cells in the SGZ of pups may not receive as much synaptic innervation as in the mature hippocampus (see section 2.2), and are thus less sensitive to neuronal activation. It was also shown that the two and three weeks old rats differ in sensitivity to pilocarpine induced-seizure, as the three weeks old rats develop SE, MFS and hilar cell loss, while the two weeks old rats do not[68]. These results demonstrate that the hippocampal environment is maturing and changing its responses to stimuli, such as seizure activity.

Further, studies have shown that prolonged seizures impair neuronal maturation and irreversibly reduce cell number, size and total brain weight[65]. As mentioned above, some pup models show hippocampal network reorganization and MFS, which form recurrent excitatory circuits that may contribute to hippocampal hyperexcitability. However, this is regarded as improbable, as long-term hyperexcitability appears after one week of FS, whereas MFS appears much later[67]. Nevertheless, one should not exclude the possibility that MFS contributes to maintain the hippocampus hyperexcitable, as it is likely that several factors are implicated in hyperexcitability. Interestingly, the majority of neurons born following seizures induced at P19 do not survive for a long period. Nonetheless, a substantial fraction of these newly born granule cells persist in the GL and become mature neurons[66]. These results suggest that seizure-induced neurogenesis at P19 may increase the susceptibility to develop chronic seizures later in life. However, it still has to be determined whether these cells function abnormally and if this is also the case for cells born after seizures induced in the first two weeks.

So far, evidence suggests that the effect of seizures on neurogenesis in the immature brain is affected by the developmental stage when the seizures are induced, the severity of seizures and the method of seizure induction. It has also been proposed that the differences in epileptogenesis are not related to differences in SE-induced neurogenesis[68]. However, it seems too premature to make such an assumption. For instance, all studies have administered BrdU several days after the first treatment to induce SE. It would be interesting to inject BrdU before the induction of seizures, simultaneously with SE induction and at several other time-points, as it has been previously

established that the S phase takes about 8 hours[70]. This would give a more accurate view of the effect of seizures over a longer period.

A study used ICV KA to induce seizures in one month old rats and three months old rats and compared the cell proliferation rates between both groups, demonstrating that there was no significant age-dependent effect[71]. However, this may be because of the small time interval between both groups. In contrast, several studies have shown that cell proliferation declines 60-80% in aged (around 18 months old) F344 rats, Sprague-Dawley rats and C57BL/6 mice[48]. This substantial decrease possibly reflects an impaired maturation, since only 14% of the newborn cells are $NeuN^+$ compared to 38-39% in the age-matched control[41]. Moreover, the percentage of DCX^+ cells is equivalent to the young adult rat, demonstrating that neuronal differentiation does not alter in the intact aged hippocampus[30, 41, 48]. In addition, while the aged hippocampus still supports the survival of newborn neurons, the processes of neuronal migration and maturation have been shown to be impaired[30, 48]. This impairment possibly results from an age-related decline in the growth factors IGF-1, BDNF, VEGF and FGF and serotonin, which promote neurogenesis (see sections 2.1 and 2.2). Furthermore, the expression of glucocortisteroids increases during old age, which may possibly contribute to decrease neurogenesis in the intact old brain[30].

Recently, it was shown for the first time that the IP KA-induced seizure model does not increase neurogenesis in 24 months old adult rats. This was not because cell proliferation was impaired, as the number of proliferating cells showed a 5.9-fold increase after SE induction. In contrast to the intact old rat brain, the epileptic old brain showed only 9% of DCX^+ cells after SE induction compared to 76 % in the age-matched intact hippocampus, suggesting that seizure activity reduces neuronal fate determination (see figure 2.7). This impaired neuronal differentiation may be due to increased expression of Musashi-1, which instructs the cells to remain either undifferentiated or differentiate into glial cells (see section 2.2). Besides, SE seems to further impair maturation, since only 6% of the newborn cells are $NeuN^+$. Interestingly, reduced neurogenesis in the aged epileptic hippocampus is associated to 92% less aberrant migrated new granule cells than in adult rats, although also 35% of the newborn neurons migrate aberrantly to the hilus (see section 2.3)[41]. Thus, because neurogenesis declines, less newborn neurons contribute to the formation of GCD and therefore it seems that in the aged epileptic brain newborn neurons are not significantly involved in maintaining GCD.

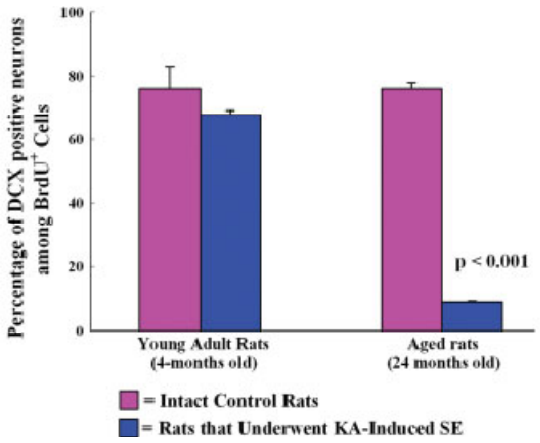

Figure 2.7: Percentage of $DCX^+/BrdU^+$ neurons in young adult rats and aged rats treated with IP kainic acid or saline. Neuronal fate determination is clearly impaired in the aged rat after KA-induced SE. (From Rao MS., et al., 2008.)

There is still controversy whether seizure severity affects neurogenesis. For instance, the IP KA model shows a significant higher decrease in cell proliferation than the ICV KA model, probably due to the higher frequency of spontaneous recurrent motor seizures (SRMS) in this KA model. In contrast, in a study where electrical stimulation was used to induce SE, different SE severities were provoked showing similar extent of cell proliferation. Likewise, milder seizures, such as kindling, also induce the same number of cells to proliferate. Based on these results, it seems that

once SE severity reaches a certain threshold the same amount of cell proliferation will be promoted. Nevertheless, the SE severity may influence the survival of newborn neurons as it determines how many neurons survive during a 4 week period[28, 53].

In conclusion, considering that the expression of proteins in the hippocampal environment of the rat pup is unlike the expression in the adult rat hippocampus and the synaptic organization is not yet finished, it is not surprising that the dentate gyrus of the young rat pup responds differently to seizure activity. It has yet to be determined exactly how seizures affect the developing hippocampus and what the long-term effects are. Throughout life, changes occur in the hippocampal environment, such as less expression of growth factors, which decrease neurogenesis. As mentioned before, the old intact hippocampus shows decreased expression of growth factors, which may further decline following SE-activity. Thus, decreased neurogenesis following SE in old aged rats is possibly due to the fact that the dentate environment does no longer support neurogenesis. As aged animals still develop SE and are even more susceptible for chemoconvulsants than younger rats, one could propose that neurogenesis is not essential to induce prolonged seizure activity in aged rats. Interestingly, it has been determined that the cell cycle takes 24.7 hours in the 10 weeks old rats compared to 16 hours in the 2-3 weeks old rats, clearly illustrating that age-dependent factors impair neurogenesis[71].

2.5 Translating Data from Rodent Models to Temporal Lobe Epilepsy Patients

The majority of the studies that investigated whether neurogenesis is involved in TLE used either the pilocarpine or kainate induced seizure model. These chemoconvulsants induce seizures that resemble the behavioural and pathophysiological aspects of TLE in humans. Nevertheless, these substances differ in their mechanism-of-action. Pilocarpine is a non-selective muscarinic agonist that induces NMDA receptor mediated changes in the morphology, membrane properties and synaptic responses of hippocampal neurons[72]. In contrast, treatment with kainic acid impairs GABA neurotransmission, possibly due to the loss of GABA receptor containing cells[73]. However, it is unknown whether these methods of seizure induction are comparable to the mechanisms underlying human TLE. The paradigm is that if the seizure models present pathophysiological changes that resemble those found in TLE, the underlying molecular and cellular mechanisms are likely also equivalent. However, this may not be entirely true, as each chemoconvulsant may affect different pathways that will lead to a similar pathophysiology, since several molecular networks may be involved in the same process. Furthermore, in these models seizures occur bilaterally, in contrast to humans where TLE is always unilateral. This clearly depicts to what extent the genetic and molecular pathways underlying epileptogenesis differ between rodent models and TLE patients.

Studies in human sample tissues also show some downsides. As discussed in the introduction, there is a lot of variability between patients as they have different genetic backgrounds, initial insults and age of epilepsy onset. Hence, at the time of surgery they will have diverse seizure severity and pathophysiology. Furthermore, there are several confounding factors as the samples come from patients with refractory epilepsy, which differ in the age at time of surgery, duration of the epilepsy period and treatment with AEDs. Therefore, when using patient samples, one has to keep in mind that the results cannot be generalized to all TLE patients. For instance, patients

with hippocampal sclerosis show changes in the expression of genes related to among others, cell structure, ion-transport and neural activity[7]. In addition, because it depicts the chronic phase of TLE, it cannot be established whether the observed molecular changes are the cause or consequence of TLE.

An alternative approach may be using neuroimaging techniques to provide indirect evidence for molecular changes in the temporal lobe of epileptic patients. For instance, the non-invasive technique, Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) that shows the distribution of metabolites in the brain, demonstrated that the metabolite N-acetylaspertate (NAA), which is only synthesized in the mitochondria of neurons, is decreased in TLE patients. This metabolite is not an indication for neuronal loss, as it was shown to decline in the undamaged contralateral hippocampus and also in the pilocarpine rat model shortly after SE induction[74]. It seems more likely that it reflects metabolic impairment[75] and therefore, neuroimaging can be a valuable tool to study neurometabolic dysfunction in TLE providing evidence for mechanisms that possibly underlie epileptogenesis such as the impaired glutamate-glutamine cycle.

Assessing whether neurogenesis is associated to epileptogenesis in TLE patients has proven to be intricate due to the factors mentioned above and to the lack of markers specific for proliferating cells and proper controls. For instance, there is evidence suggesting that the proliferation rate in patients is much lower than in rodent models. On the other hand, it has been shown that Vimentin and Nestin, both markers for putative stem cells, are upregulated in young patients[56]. Further, the PolySialylated Neural Cell Adhesion Molecule (PSA-NCAM), a marker for immature neurons, was also found to be upregulated. However, this marker is not very specific as it may be expressed in different cells, such as the precursor cells[55]. Furthermore, seizure activity may upregulate the expression of these biomarkers without necessarily relating to actual changes in the number of stem cells, cell division or immature neurons[64].

Several studies found no evidence for enhanced neurogenesis in TLE patients above two years of age, as the expression of markers for immature neurons was not increased. These findings may be contradicting because of the larger differences in patient ages and number of patients in the sample group used[18]. Interestingly, it has been shown that patients with febrile seizures (FS) and hippocampal sclerosis (HS) have significantly more cells positive for Musashi-1 and $p27^{Kip1}$, which is a division repressor, than FS^{-} HS^{-} patients. These results suggest that newborn cells do not differentiate into neurons in the FS^{+} HS^{+} patients, as Msi-1 activates the Notch pathway (see section 2.2)[55], proposing that there is a similarity between the chronic stage in the rodent models and FS^{+} HS^{+} patients. Hence, the data from TLE patients does not support the findings from rodent models following acute epilepsy, possibly due to the lack of appropriate markers and to the phase at which the samples were taken.

Some studies reported that TLE patients with GCD had significantly more neurogenesis than patients without GCD. Moreover, there is strong evidence that Reelin expression is downregulated in the hippocampus of some TLE patients, and correlates with the extent of GCD[18]. However, another study did not find $Msi\text{-}1^{+}$/$NeuN^{+}$ cells in the regions of granule cell dispersion [20, 55]. It has also been found that DCX expression is lost, suggesting that GCD is caused by aberrant migration of differentiated cells. One of the reasons that these results do not correlate with the data obtained in rodent models may be that in patients, the studies are carried out in the chronic phase while the data from rodent models represents the acute phase. Furthermore, TLE patients are genetically more heterogeneous than rodent models, which may explain why the results are so divergent from each other. Hence, although strong evidence supporting a role for

neurogenesis in epileptogenesis in TLE is still lacking, it cannot be ruled out that at some point neurogenesis may play a role in epileptogenesis.

It is difficult to determine to what extent the data from SE models can be translated to TLE patients. As discussed above, the SE models are not very representative of human TLE, yet there is no doubt that rodent models provide an invaluable tool to clarify the molecular mechanisms underlying processes that are likely conserved between the two species. Further, it has an advantage in allowing complex processes to be investigated in a simpler model under standardized conditions, providing insights into potential mechanisms underlying TLE.

2.6 Summary and Perspective

So far, evidence suggests that neurogenesis temporarily increases during the acute phase and returns to baseline before substantially declining during the chronic phase (see section 2.2). As discussed before, interpreting the data from studies attempting to assess whether neurogenesis is upregulated after SE induction in the IP and ICV KA models can be influenced by the extent of hippocampal damage and by the time-point of BrdU injection. Therefore, the method of SE-induction and the time-point when BrdU is administered should be carefully chosen. Further, it has become clear that neurogenesis is also influenced by age-dependent mechanisms, as it decreases with age and is not enhanced by seizure activity. It has also become evident that due to age and/or disease related changes the environment of the dentate gyrus may no longer support or promote neurogenesis.

It is well known that having febrile seizures early in life is an important risk factor for developing TLE at a later time point. Interestingly, studies have shown that the immature hippocampus reacts differently in response to SE induction in comparison to adult rats, suggesting that in pups aberrant neurogenesis may play a more important role in epileptogenesis (see section 2.4). It also raises the possibility that an early seizure episode may change the hippocampal environment in such a way that neurogenesis may contribute to epileptogenesis at a later time point. This was demonstrated by a study where adult rats (P66) showed a 25% increase in $BrdU^+$ compared to the aged matched control after hyperthermia-induced seizures at P10[76]. This result may be significant to TLE patients as this seizure model mimics TLE more accurately. It remains to be determined whether these newborn neurons contribute in any extent to epileptogenesis.

Currently, the results suggest that neurogenesis does not play an essential role in epileptogenesis in adult rodents (see section 2.3). Several lines of evidence imply that newborn neurons have an impaired cell fate determination and may be involved in the formation of GCD. However, additional studies are required to determine to what extent impaired cell differentiation would contribute to epileptogenesis and to what extent the newborn neurons may contribute to the formation of GCD. It is also important to establish during which phase of the epileptogenic period the newborn neurons may contribute to GCD or MFS. This could be done by quantifying the proliferation, apoptosis and survival rate of BrdU positive cells after IP KA-induced SE in adult rats.

So far, no evidence has been found to suggest that neurogenesis also increases in TLE patients. As discussed before, one of the reasons why studies on brain tissues from TLE patients show contradicting results is because of when the studies are performed during the epileptogenic period. This poses the problem that one does not know if the changes observed are the cause or

consequence of the epileptogenic process. Neuroimaging studies have shown that neurometabolic dysfunction in the dentate gyrus occurs since an early phase in epileptogenesis. Based upon current knowledge from neuroimaging studies and animal models, one could propose that if neurogenesis increases in TLE patients, it would not be likely to function as an adaptive mechanism protecting the hippocampus. It seems likely that as the existing neurons are affected by the extensive molecular and cellular changes that occur in the hippocampal environment, the newborn neurons would also be affected and may therefore be involved in epileptogenesis. It is crucial to determine to what extent the newborn neurons may be affected by impaired neuronal differentiation or contribute to GCD and MFS formation.

As mentioned in the previous section, the rodent models used to study epilepsy are not very representative of TLE in humans. Developing transgenic animal models may improve the face validity of the model and help understand the effect of human gene mutations on neurogenesis or the development of chronic epilepsy. Imaging techniques still provide restricted research possibilities, so before becoming a regular research tool its resolution and markers have to improve. Without a doubt, progress in animal models and imaging techniques will advance our understanding of the TLE epileptogenesis.

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