# HABILITATION A DIRIGER DES RECHERCHES

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Titre de la thèse : *« Reprogrammer l'identité cellulaire au sein du système nerveux central: Une nouvelle approche pour réparer le cerveau »*

## Résumé

1-PhD Work

The Mesio-Temporal Lobe Epilepsy (MTLE) syndrome appears to be one of the most pharmacologically resistant forms of human epilepsy. This syndrome is characterized by an initial precipitating event, followed by a latent period (i.e. epileptogenesis) before the appearance of spontaneous recurrent seizures in the hippocampus. Granule cell dispersion (GCD), a widening of the granule cell layer in the hippocampus, is often evidenced in the sclerotic hippocampus of MTLE patients. Little is known about the mechanisms underlying epilepsy development and GCD development. The aims of the my PhD were to (i) examine the role of the Brain-Derived Neurotrophic Factor (BDNF) in the development of MTLE, and (ii) to investigate the molecular mechanisms underlying GCD formation. These questions were addressed in a mouse model of drug-resistant MTLE, obtained by intrahippocampal injection of kainate (KA) in the adult mouse, which induces focal epileptic seizures and GCD, comparable to the changes observed in the human disease.

First, we evidenced a long lasting and significant overexpression of BDNF in dentate granule cells in parallel with epileptogenesis. To determine whether such a BDNF increase could influence epileptogenesis via its TrkB receptors, we examined the consequences of (i) increased or (ii) decreased TrkB signaling on the time course of epileptogenesis, in transgenic mice overexpressing (i) the TrkB full-length or (ii) the truncated TrkB-T1 receptors of BDNF. We showed that increased TrkB signaling facilitated the progression of epileptogenesis and aggravated the severity of hippocampal seizures. On the contrary, epileptogenesis was delayed in transgenic mice with reduced TrkB signaling. In addition, mice with enhanced TrkB signaling exhibited an increased number of generalized seizures, thus suggesting increased seizure susceptibility and neuronal excitability in these mutants. In contrast, different levels of TrkB signaling did not influence the extent of GCD and the recurrence of hippocampal recurrent seizures during the chronic phase. Taken together, these data show that the increase in BDNF-mediated TrkB signaling promotes epileptogenesis in the KA mouse model of the MTLE syndrome.

Next, we investigated whether GCD could result from an increased dentate neurogenesis followed by an abnormal migration of the newly generated granule cells. Ipsilateral GCD progressively developed following KA injection and was paralleled by a gradual decrease in the expression of doublecortin, a marker of newly generated granule cells, in the dentate subgranular layer. Labeling with bromodeoxyuridine showed an early and transient increase in mitotic activity in the dentate gyrus of the KA-injected hippocampus, which gave rise to microglial cells and astrocytes but not

to new neurons. Moreover, at later time points, there was a complete cessation of mitotic activity in the injected hippocampus (where GCD continued to develop), but not on the contralateral side (where no GCD was observed). Next, we investigated potential changes in the expression of reelin, which is a critical protein in the control of neuronal migration. We evidenced a significant decrease in reelin mRNA synthesis in the injected hippocampus in parallel with GCD development. Finally, the neutralization of reelin by application of the CR-50 antibody induced GCD in naïve adult mice. Taken together, these results show that GCD does not result from increased neurogenesis but reflects a displacement of mature granule cells, likely caused by a local reelin deficiency.

## 2-Postdoc work

During my PhD I gained a strong interest in studying neurogenesis in the context of neurological diseases. Therefore, I decided to join the lab of Prof Dr Magdalena Götz (Institute of Stem Cell Research, Munich, Germany), who is a world-leading expert in this field. Injury to the human central nervous system (CNS) is devastating because our adult mammalian brain has little, if any, intrinsic regenerative capacity to replace lost neurons and induce functional recovery. Hence acute CNS injury and chronic neurodegenerative diseases are associated with irreversible loss of neurons, ultimately leading to permanent functional deficits and neurological disability. The main goal of regenerative medicine for brain repair is to replace lost neurons using cell-based strategies in order to restore lost functions. As a postdoc in the Götz lab, I explored a unique and innovative strategy aiming at reprogramming reactive glial cells, residing at the injury site, into clinically relevant neurons (*i.e. induced neurogenesis*), with the underlying rationale to recruit glia as an endogenous cellular source for brain repair. In addition we aimed at exploring the exciting question in the field of reprogramming whether glial cells could be directly reprogrammed into functional induced neurons (iNs).

**First, we showed that astroglia from the postnatal mouse cerebral cortex can be reprogrammed in vitro to generate functional, synapse-forming neurons by forced expression of neurogenic transcription factors (TFs).** Using retroviral vectors encoding Neurogenin2 (Neurog2), a TF known to instruct the generation of glutamatergic neurons during development, we instructed with high efficiency the reprogramming of astroglia into βIII tubulin+ and MAP2+ neurons (70% of Neurog2-transduced astroglia). In sharp contrast, astroglia transduced with a control retrovirus remained in the glial lineage. Using patch-clamp recordings we showed that (i) astroglia-derived iNs acquired the true hallmarks of functional neurons including repetitive action potential firing, and that (ii) 60% of iNs specifically matured into synapse-forming glutamatergic neurons that gave rise to glutamatergic networks. Importantly, we confirmed the astroglial origin of iNs by using a genetic fate-mapping strategy using GLAST::CreERT2/GFP mice, in which GFP reporter expression can be induced specifically in astroglia and is then maintained in their progeny. This strategy confirmed that Neurog2 instructed GFP-fate-mapped astroglia to differentiate into GFP+ neurons. Importantly, the neurotransmitter identity of astroglia-derived iNs could be controlled by selective expression of distinct neurogenic fate determinants: Forced expression of Dlx2 and/or Ascl1, TFs known to instruct the genesis of GABAergic neurons during development, induced a fate switch of astroglia towards GABAergic interneurons. Patch-clamp recordings revealed that depolarisation of these iNs evoked bicuculline-sensitive GABAergic autaptic responses, thereby demonstrating their GABAergic identity. **This was the first time that the generation of functional iNs could be achieved by direct conversion across cell lineages induced by a single neurogenic TF.** We also showed that high levels of oxidative stress impede direct neuronal reprogramming and found evidence that co-expression of Bcl-2 with reprogramming TFs, anti-oxidative treatments and inhibitors of ferroptosis improve glia-to-neuron conversion.

A major challenge was the translation of the above in vitro findings into the adult mouse brain in vivo and following injury. We showed that glial cells proliferating in the cortex of adult mice in response to stab wound injury could be directly reprogrammed in vivo into DCX+ iNs by forced expression of Ascl1 and Sox2 (30% of Sox2/Ascl1 co-transduced cells). Strikingly, forced expression of Sox2 alone also induced conversion of the transduced glial cells into DCX+ neuroblasts that progressively matured over time as revealed by NeuN expression. To investigate the origin of the cells converted into iNs, we used a genetic fate-mapping strategy using an inducible Sox10-iCreERT2/GFP transgenic mouse line, and showed that the vast majority of iNs derived from the conversion of NG2 glia. Finally, patch-clamp recordings in acute slices revealed that iNs elicited action potentials and received spontaneous synaptic inputs from endogenous neurons neighboring the injury site. This indicated that iNs were capable of assembling a functional postsynaptic compartment. They were recognized by endogenous neurons as functional synaptic targets and were incorporated to some extend into local neuronal circuits. **Together our results show that glia-to-neuron conversion can**

**be achieved in vivo in the injured adult brain, opening new avenues toward the use of endogenous glial cells for brain repair.**

A key issue is whether the adult human cortex contains cells amenable to neuronal reprogramming. Importantly, we showed that adult human non-neuronal cells, once isolated in vitro from cortical tissues surgically resected from human patients, could be instructed to generate NeuN+ and GABA+ iNs following forced expression of Ascl1 with Sox2. Moreover, these iNs were able to fire action potentials and received functional synaptic contacts, indicating their capability of integrating into neuronal networks. Finally, combining fluorescence-activated cell sorting (FACS) analysis and time-lapse video microscopy of FACS-sorted cells, we showed that iNs derived from human cells expressing the hallmarks of pericytes. **Our results raise the possibility of conversion of endogenous cells in the adult human brain to induced neuronal fates.**

## 3-Current work at CNRS

Since pioneering in vitro studies, considerable progress has been made in instructing in vivo reprogramming of astroglia, NG2 glia or microglia to generate functional iNs of various phenotypes within the adult mouse cortex, striatum and spinal cord. While glia-to-iN reprogramming holds promise as neuron-replacement strategy, a critical question is now whether iNs are endowed with the capability of promoting functional recovery in pathological contexts. In the present study, we addressed this question in the context of MTLE, which is associated with hippocampal sclerosis including severe neuronal loss and reactive gliosis, as well as a degeneration of hippocampal GABAergic interneurons which was suggested to promote the epileptic state. We hypothesized that regeneration of GABAergic neurons by in vivo lineage reprogramming of reactive glial cells could represent an innovative approach to reduce seizures in MTLE. Using a well-established mouse model of chronic MTLE (i.e. MTLE mice, obtained by intrahippocampal KA injection), we aimed to induce reprogramming of reactive glial cells into iNs that i) acquire a GABAergic identity, ii) functionally integrate into epileptic networks and restore lost inhibitory transmission, and iii) ultimately reduce hippocampal seizures.

We followed a retrovirus strategy to target expression of the reprogramming genes Ascl1 and Dlx2 to reactive glia, known to proliferate in the sclerotic hippocampus. Importantly, we and others previously showed complete cessation of adult dentate neurogenesis in the MTLE hippocampus resulting from depletion of neural stem cells, that we have confirmed here by supplying BrdU for 3, 5, or 7 days post KA injection (dpKA). Virtually all BrdU+ cells expressing DCX had disappeared by 5 dpKA contrary to saline-treated mice. Thus, we selected 5 dpKA for retroviral injection as a suitable time point to exclusively target reactive glia in absence of physiological neurogenesis. Following injection of a control retrovirus encoding DsRed-only in the sclerotic hippocampus, transduced cells essentially comprised NG2 glia, reactive astroglia and microglia that remained in their glial lineage. Importantly, none of the transduced cells expressed neuronal markers nor exhibited granule cell morphology, thus demonstrating that the retrovirus only transduces reactive glia devoid of neurogenic potential.

In contrast, retrovirus-mediated expression of Ascl1/Dlx2 (together with DsRed) induced reprogramming of hippocampal glia into iNs with high efficiency: 70% of transduced cells expressed the immature neuron marker DCX and exhibited an immature neuron morphology at 7 days post infection (dpi). Consistent with progressive maturation, iNs expressed MAP2 and NEUN after 6 weeks, and exhibited complex neuronal morphologies extending several branched processes. To demonstrate genuine glia-to-neuron conversion, we initially labeled dividing glia with BrdU prior to retrovirus injection. This revealed a very high rate of iNs showing both NEUN and BrdU labeling, demonstrating their de novo generation. We next asked whether iNs acquired a GABAergic identity and repeated the same experiments using GAD67-GFP mice, from which glia-derived GABAergic neurons will turn on GFP under the GAD67 promoter. 75% of iNs had differentiated into GABAergic neurons after 6 weeks, as revealed by GFP reporter expression. We also found that a significant fraction of iNs expressed NPY, VIP or SST, thus revealing acquisition of interneuron subtype-specific features.

Next we examined the synaptic integration of iNs within endogenous networks by using rabies virus (RABV)-mediated retrograde synaptic tracing. Hippocampal glia were co-transduced with retroviruses encoding Ascl1/Dlx2 and the TVA receptor for the EnvA-pseudotyped RABV and its glycoprotein G. Six weeks later, GFP-encoding RABV selectively targeted the TVA-expressing iNs and spread to their presynaptic partners. iNs received innervation from a large amount of local granule cells within the epileptic dentate gyrus. We also observed a moderate but consistent innervation by long-range projection neurons from remote brain areas such as the entorhinal cortex and the mammillary bodies. Next, we found that iNs extended axons creating dense networks through the MTLE dentate gyrus with axonal varicosities

impinging on granule cells and en passant synaptic boutons contacting successive granule cell dendrites, thus suggesting that iNs form synapses with granule cells that likely provide critical substrates for seizures.

Next, we performed patch-clamp recordings in slices to assess whether glia-derived neurons acquired properties of functional neurons. GABAergic iNs were capable of repetitive AP firing and exhibited firing patterns reminiscent of low-threshold spiking interneurons. To assess whether iNs form GABAergic synapses with granule cells, we followed an optogenetic strategy and initially targeted to reactive glia co-expression of the reprogramming genes and ChannelRhodopsin2 (ChR2). ChR2-expressing iNs were stimulated by blue light pulses, while granule cells were recorded. Optical stimulation of iNs induced their AP firing and evoked Inhibitory Post Synaptic Potentials (IPSPs) in granule cells that were blocked by gabazine, a selective antagonist of $GABA_A$ receptors, thus demonstrating that iNs establish GABAergic synapses onto granule cells.

Next, we investigated whether GABAergic iNs reduce spontaneous hippocampal seizures during the chronic phase of the disease by recording EEG activity. We found that the number of spontaneous seizures was significantly decreased in MTLE mice injected with the Ascl1/Dlx2-encoding retrovirus compared to control virus-injected animals. In addition, Ascl1/Dlx2-injected mice spent significantly less time in seizures compared to controls. Together, these data demonstrate that in vivo reprogramming of hippocampal glia into GABAergic neurons decreases chronic seizure activity in MTLE mice.

Next, to further demonstrate a functional impact of GABAergic iNs on seizures, we followed an independent approach. Cortical astroglia isolated in vitro were transduced with the retrovirus encoding Ascl1/Dlx2 directly before being grafted in the epileptic hippocampus and allowed for reprogramming in vivo. Like resident hippocampal glia, grafted astroglia were converted with high efficiency into functional GABAergic neurons showing some degree of specification toward distinct interneuron subtypes. Graft-derived neurons also functionally integrated within endogenous epileptic networks and established GABAergic synapses onto granule cells. EEG monitoring revealed that these GABA neurons derived from grafted glia also led to a significant reduction in the number of seizures. Together, these grafting data provide additional evidence for the ability of glia-derived GABAergic neurons to reduce chronic seizure activity. We observed that 40% of glia-derived iNs were highly active during the chronic phase, suggesting that the overall level of iN activity could be further increased to enhance their seizure-suppressing effects.

To address this, we followed a chemogenetic approach and initially targeted to astroglia co-expression of Ascl1/Dlx2 and the excitatory DREADDs hM3Dq, thereby allowing subsequent selective activation of glia-derived iNs by CNO treatment. We first showed in control MTLE mice that CNO injection on its own had no significant effects on number and cumulative duration of seizures. In contrast, CNO-mediated activation of hM3Dq+ GABAergic iNs almost entirely suppressed the remaining seizures, below the reducing effects of iNs in absence of CNO in the same mice. In fact, only residual low-frequency and isolated spikes remained visible after CNO. Together, this reveals that the level of iN activity is crucial for the therapeutic impact of iNs on seizures.

**Taken together, we show for the first time that Ascl1/Dlx2 efficiently reprogram hippocampal reactive glia into GABAergic interneurons that functionally integrate into epileptic networks and establish inhibitory synapses onto granule cells primarily involved in hippocampal seizures. Our study provides first evidence that GABAergic iNs reduce chronic seizure activity, thus uncovering glia-to-neuron reprogramming as a potential disease-modifying strategy to control seizures in therapy-resistant epilepsy.**