SUMMARY

Chapter I. Postdoctoral Scientific Activity. The present work synthesizes the results of my postdoctoral scientific activity, conducted through direct affiliation as a research fellow or through close institutional collaborations with prestigious German research centers, such as the German Cancer Research Center (DKFZ) and the Institute of Anatomy and Cell Biology at Heidelberg University. This work integrates two major pathological directions: renal immunopathology (transplant and metabolic) and neurobiological dysfunctions in Alzheimer's disease models. Through a multidisciplinary methodological approach - combining classical histopathology and immunohistochemistry with high-resolution morphological analysis and advanced molecular biology - these studies have enabled not only a detailed phenotypic description but also the identification of strategic biomarkers and molecular targets for novel therapeutic interventions, tailored to the specific nature of each investigated pathology.

I.A. Modulation of PPARγ and LXR Nuclear Receptors in Renal Transplantation and Metabolic Pathology

The first section includes studies that build upon and extend the scientific foundation established during my doctoral thesis. This research direction further explored the cellular and molecular mechanisms of renal fibrosis, with a particular focus on inflammatory dynamics and the critical role of macrophages. The investigation centered on the subfamily of nuclear receptors that form heterodimers with RXR, utilizing advanced rodent experimental models (rats/mice).

1. **PPARγ in Experimental Renal Transplantation**. The study monitored the transcriptional dynamics of the extracellular matrix, highlighting the specific inhibition of biglycan and decorin proteoglycan synthesis by the PPARγ agonist in the renal allograft, independent of its metabolic effects.
2. **LXR in Experimental Renal Transplantation.** This research analyzed how the activation of LXRα and β isoforms protects transplanted kidneys from long-term degradation. The methodology involved the use of the GW3965 agonist in Fisher-Lewis rat models and transgenic mice overexpressing LXRα in macrophages.
3. **LXR in Experimental Diabetic Nephropathy.** The study utilized LDL-receptor deficient (hyperlipidemic) mice with streptozotocin-induced diabetes, alongside transgenic lines overexpressing LXRα in macrophages. This approach was complemented by pharmacological intervention with the GW3965 agonist and in vitro analyses on macrophages exposed to glycated/acetylated LDL. The results revealed a critical link between lipid metabolism and inflammation, an interface modulated through the activation of LXR receptors.

I.B. Investigation of Inhibitory Synapse Dysfunction in Alzheimer's Disease (AD) Models. The second major direction of my research activity focused on the study of inhibitory synapses, examined both through the dynamics of parvalbumin-positive (PV+) interneurons and as strategic therapeutic targets in APP/PS1 transgenic mouse models during the early and late stages of amyloidogenesis. The analysis of molecular mechanisms specifically targeted the role of gephyrin as a fundamental scaffolding protein in the organization of the inhibitory postsynaptic compartment.

1. **Pathological Profile of GABAergic Inhibitory Synapses.** This section includes studies focused on the molecular and electrophysiological mechanisms through which the amyloid cascade disrupts the integrity of inhibitory circuits in APP/PS1 mice in the absence of pharmacological intervention.
   - **Biphasic Alteration of Inhibitory Synapses:** Pathological progression was monitored in the transgenic mouse model, evaluating the dysfunction of GABAergic transmission from presymptomatic stages (1–3 months) to advanced neurodegeneration (8–12 months). The methodology integrated high-resolution confocal microscopy (for the quantification of gephyrin

and the $GABA_A$ receptor γ2-subunit) with biochemical analysis used to determine changes in the solubility and expression of gephyrin isoforms.

- **Vulnerability of PV+ Interneurons.** Advanced morphometric and electrophysiological analysis techniques (sharp wave-ripple complexes and γ-oscillations) were included to assess the integrity of inhibitory circuits in the presymptomatic phases (1–3 months) of the APP/PS1 model, facilitating the direct correlation of structural changes in PV+ interneurons with hippocampal information flow dysfunction.
- **Role of the p35/CDK5 Signaling Axis:** It was investigated whether the p35/CDK5 signaling pathway is involved in the increased perisomatic phosphorylation of gephyrin and the enhancement of GABAergic inhibition in hippocampal subregions of young APP/PS1 mice. In vivo analyses were correlated with in vitro studies on primary hippocampal neurons, where the use of shRNA for p35 gene silencing allowed for the validation of this protein's role in regulating gephyrin phosphorylation.

2. **Therapeutic Modulation via Artemisinins.** This section highlights original contributions regarding the repurposing of artemisinins - conventionally used in antimalarial therapy - as neuroprotective agents capable of interfering with AD progression through various mechanisms of action.
   - **Early-stage effect: Regulation via phosphorylation.** Direct observation in living models (3-month-old APP/PS1 mice) was combined with precise *in vitro* genetic manipulation (site-directed mutagenesis) to demonstrate that artemisinin stabilizes GABAergic synapses by activating the p35/CDK5 enzymatic cascade.
   - **Late-stage effect: Modulation of Aβ metabolism and recovery of GABAergic inhibitory signaling.** The effects of artemisinin and artesunate were evaluated in advanced stages of the disease (12-month-old APP/PS1 model). It was demonstrated that, by inhibiting BACE1 enzyme activity, artesunate treatment successfully reduces the production of toxic amyloidogenic fragments, such as CTFs, while simultaneously restoring anchoring protein levels (such as gephyrin) and $GABA_A$-γ2 receptor density at the postsynaptic membrane.
   - **Modulation of the glycinergic network.** The protein levels and subcellular localization of glycine receptor α2 and α3 subunits were analyzed in the hippocampus of APP/PS1 mice at different AD stages, alongside the influence of artesunate treatment.

Chapter II. Development Plans for Professional, Scientific, and Academic Career Evolution. This evolution plan aims to consolidate a prestigious academic career at the intersection of cellular and molecular biology and the study of neurodegeneration mechanisms. Professionally, the strategy focuses on enhancing the quality of the teaching process by integrating a student-centered educational model based on mentorship and innovation. Concurrently, the plan seeks to strengthen my academic status by obtaining the habilitation degree and exercising the role of a doctoral supervisor.