Neuro gune

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Abstract Book

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Scientific Committee

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2. PROGRAMME

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3. KEYNOTES

Opening Keynote

Roelof HUT
Groningen Institute for Evolutionary Life Sciences,
University of Groningen (NL)

"The temporal niche arms race: an evolutionary view on biological rhythms"

Closing Keynote

Aude PANATIER
INSERM, Bordeaux (FR)

"D-serine's journey, from metabolism to memory"

4. SPONSORS

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5. ORAL PRESENTATIONS

Oral Communication 01

Clinical Neuroscience / Neurodegenerative diseases

RESTORATION OF SPLICING AND ASSOCIATED MITOCHONDRIAL ALTERATIONS USING PERSONALIZED ANTISENSE OLIGONUCLEOTIDES FOR A DEEP INTRONIC SPG7 FOUNDER VARIANT

Hereditary Spastic Paraplegia Type 7 (SPG7) is an incurable late-onset neurodegenerative disorder with phenotypes ranging from pure spastic paraplegia to ataxia. Our group has identified a cohort of 11 patients from the Basque Country and neighbouring areas with a deep intronic mutation in SPG7 gene (c.286+853A>G). Combining Oxford Nanopore long-read sequencing and short-read sequencing of seven patients, we have established a shared haplotype in Northern Spain, suggesting this mutation originates from a common ancestor. At the molecular level, this variant activates a cryptic exon that introduces a premature stop codon, resulting in SPG7 loss of function and impairing the assembly of the mitochondrial m-AAA protease complex (SPG7-AFG3L2), which is essential for mitochondrial proteostasis. Personalised antisense RNA therapies have recently proven to be safe and effective genetic therapies for several neurodegenerative diseases. Following this strategy, we have developed eight splice-modulating antisense oligonucleotides (ASOs) specifically designed to block the inclusion of the SPG7 cryptic exon. Our lead candidate successfully restored physiological mRNA processing and normalized protein expression levels in patient-derived cells. Furthermore, this treatment achieved a significant downstream rescue by restoring the assembly of the SPG7-AFG3L2 mitochondrial complex. Future work will focus on evaluating the efficacy of the lead ASO in iPSC-derived motor neurons and cerebellar organoids. Additionally, we aim to conduct longitudinal clinical assessments of the patients and identify biomarkers capable of evaluating disease progression and monitoring therapeutic response. Our ultimate goal is to establish a comprehensive preclinical and regulatory framework to obtain AEMPS approval for intrathecal administration of the therapy, providing a personalized treatment for patients carrying this founder mutation.

Authors

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Oral Communication 02

Experimental Neuroscience / Glial biology and neuron-glia interactions

MICROGLIAL PHAGOCYTOSIS ACTIVELY REPROGRAMS METABOLISM AND INFLUENCES TUMOR AND NEURONAL CELL SURVIVAL

Microglia are the resident macrophages of the central nervous system (CNS) that maintain brain homeostasis by phagocytosing, for example, apoptotic or necrotic cells (efferocytosis). Recent results in the lab demonstrated that phagocytosis is not only the clearance of debris. Using single-cell RNA sequencing and metabolomics we uncovered an unexpected oxidative stress in post-phagocytic microglia, along with catabolic decline, mitochondrial remodeling, elevated galectin-3 expression, and increased polyamine production. These results suggest that post-phagocytosis events could impact the phagocytes themselves or the surrounding cells. First, we studied whether phagocytosis affects the phagocyte function in a model of glioblastoma,

subjected to two consecutive doses of radiotherapy to induce apoptosis of tumor cells. We determined that, despite continued apoptotic challenges, tumor microglia and macrophages cells were able to overcome the stress associated with phagocytosis, suggesting that the changes were adaptive and intended to maintain phagocytic efficacy. Next, we studied the effect of phagocytosis on glioblastoma U251 cells in vitro, and found that the phagocytosis secretome inhibits its survival, an effect that is enhanced when phagocytic microglia are cultured with an inhibitor of the synthesis of the polyamine spermidine. These findings are consistent with those previously obtained in the laboratory where we observed that microglia phagocytosing apoptotic newborn neurons control adult neurogenesis through the phagocytosis secretome. Together, these results reveal that microglial phagocytosis triggers adaptive metabolic and transcriptional changes that preserve their functionality while actively shaping the CNS environment through the secretome, influencing the survival of neighboring cells, including tumor and neuronal populations.

Authors

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Oral Communication 03

Experimental Neuroscience / Glial biology and neuron-glia interactions

ASTROCYTIC INSULIN RECEPTOR ABLATION PRECIPITATES ALZHEIMER'S DISEASE ONSET

Insulin is a key regulator of glucose metabolism, but its role in the central nervous system remains incompletely understood. While neuronal insulin signaling has been widely studied, the function of insulin in astrocytes—cells that comprise more than half of the brain and actively regulate neuronal activity, synaptic function, and metabolic and structural homeostasis—is still unclear. Brain insulin resistance is emerging as a central feature of Alzheimer's disease (AD), yet the contribution of astrocytic insulin signaling to disease related network dysfunction has largely been overlooked. In this scenario, our study investigates the impact of astrocytic insulin receptor (IR) deletion on AD progression, aiming to uncover how disrupted astrocyte metabolism affects neuronal circuits and cognition.

Our findings indicate that astrocytic IR depletion increases mortality rates and accelerates AD pathology in APP/PS1 mice, leading to cognitive impairments as early as 4 months of age. Notably, IRΔGFAP mice exhibit astrocyte morphological alterations distinct from typical AD-associated astrogliosis, without significant effects on amyloid or Tau pathologies. Furthermore, ex vivo Ca^{2+} imaging revealed aberrant dynamics in IRΔGFAP astrocytes, with more frequent spontaneous events but weaker and less diffuse evoked responses, reflecting a breakdown of astrocytic network integrity. Consistently, in vivo electrophysiological recordings demonstrated spontaneous hippocampal neuronal hyperexcitability and impaired circuit recruitment, further supporting the notion that astrocytic IR depletion disrupts astrocyte-neuron communication and exacerbates the AD-related network dysfunction.

These results highlight astrocytic IR as a potential therapeutic target for AD, offering an approach that surpasses classical pathological markers while providing new perspectives to tackle this devastating disease which undeniably requires innovative solutions.

Authors

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Oral Communication 04

Novel Tools and Methods / Biomarkers and diagnostic technologies

MUSCLE SPATIAL LIPIDOMICS REVEALS EARLY DIAGNOSTIC SIGNATURES OF ALS IN PRESYMPTOMATIC SOD1G93A MICE

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive motoneuron (MN) degeneration, leading to progressive muscle weakness and paralysis. In the absence of a general cure, ALS diagnosis remains challenging, often requiring up to one year for confirmation. The skeletal muscle is now recognized as an early pathogenic driver rather than a passive bystander in disease. Using lipid imaging mass spectrometry (LIMS) on the Tibialis anterior (TA) muscle of hSOD1G93A mice across disease stages, we identified fiber-type-specific and sex-dependent spatial lipid remodeling. Remarkably, lipid alterations occurred as early as the presymptomatic stage, prior to clinical symptom onset or MN loss. Specifically, LIMS accurately distinguished fast-twitch oxidative-glycolytic (type IIA) from fast-twitch glycolytic fibers (type IIB/IIX) in both wild-type and mutant mice, confirming the preferential susceptibility of type IIA and IIB fibers. Indeed, muscle lipidome analysis showed that presymptomatic mutant mice lose physiological lipid signatures while acquiring mutation-exclusive alterations. Furthermore, lipid profiles differed between female and male mice, highlighting sex-dependent variations that still allow discrimination of mutant mice at the presymptomatic stage. Finally, lipid alterations due to the disease were also detected in the serum, identifying a 4-lipid panel shared with muscle that accurately classified presymptomatic mice. Together, spatially resolved muscle lipidomics, at the fiber-type level and in a sex-dependent context, establishes lipid remodeling as an early event in ALS pathogenesis, unveiling novel mechanisms and providing candidate tissue and circulating biomarkers for early diagnosis and disease monitoring.

Authors

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Oral Communication 05

Experimental Neuroscience / Molecular and cellular neuroscience

EPITRANSCRIPTOMIC TRIAGE ORCHESTRATES BIPHASIC SYNAPTIC REMODELING AFTER HSP90 INHIBITION

Synaptic plasticity and memory formation require rapid and coordinated changes in neuronal protein expression. Among the mechanisms regulating these processes, dynamic m6A RNA methylation has emerged as a key epitranscriptomic regulator of neuronal function. However, how neurons use this system to adapt and recover from severe cellular stress remains poorly understood. Here, we identify an epitranscriptomic remodeling program triggered by acute HSP90 inhibition with radicicol.

Within 24 hours of treatment, neuronal transcripts segregate into distinct response groups characterized by opposite changes in RNA abundance and m6A methylation. Synaptic-related transcripts display reduced RNA levels together with increased m6A modification, whereas transcripts associated with proteostasis and translation show the opposite pattern. This early transcriptional and epitranscriptomic reorganization precedes a later remodeling phase marked

by selective synaptic protein synthesis and increased expression of the m6A readers YTHDF1 and YTHDF2.
At the functional level, neurons initially exhibit disrupted network activity followed by progressive recovery over subsequent days. Pharmacological experiments further revealed that the delayed structural reorganization requires de novo protein synthesis and depends on ERK and PKC signaling pathways.
Importantly, chronic intracerebroventricular administration of radicicol in vivo enhanced Barnes maze learning in two independent cohorts, supporting the idea that this remodeling process reflects active circuit adaptation rather than passive recovery. Consistent with this, the early in vitro molecular signatures predicted later transcriptional changes observed in the hippocampus of behaving mice.
Together, these findings define an epitranscriptomic framework for the neuronal response to HSP90 inhibition and support a model in which early RNA remodeling contributes to long-term synaptic and behavioral adaptation.

Authors
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Oral Communication 06

Novel Tools and Methods / Biomarkers and diagnostic technologies

SELECTED BIOMEDICAL APPLICATIONS IN FAST VOLUMETRIC IMAGING USING LIGHT-SHEET MICROSCOPY

Light-sheet fluorescence microscopy (LSFM) operates by illuminating the sample with a thin sheet of excitation light, while the emitted fluorescence is captured through a detection objective positioned perpendicular to the illumination plane. This configuration enables highly efficient excitation and signal collection. As a result, the light exposure on the specimen is minimized, leading to reduced photobleaching and consequently lower phototoxicity.
LSFM has emerged as a powerful approach for rapid three-dimensional imaging of biological specimens. In this work, I will describe our efforts to achieve fast volumetric imaging tailored for high-throughput applications, utilizing a fluidic setup.
In the second part, I will introduce an LSFM system designed for high-speed volumetric imaging that employs an electrically tunable lens (ETL). This configuration enables dynamic focusing and is applied to monitor spontaneous calcium (Ca^{2+}) activity, as indicated by GCaMP fluorescence signals.
Finally, I will present an approach that integrates wavefront coding (WFC) with machine learning in an LSFM framework, enabling the visualization of the three-dimensional dynamics of sperm flagellar motion.

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6. POSTERS

6.1 TOPIC 01: CLINICAL NEUROSCIENCE

Reference number: T01-1

NEURONAL PENTRAXINS, AGE-RELATED NEUROPATHOLOGIES, AND COGNITIVE DECLINE IN OLD AGE

Neuronal pentraxins are key regulators of excitatory synapse function, contributing to the clustering and trafficking of AMPA-type glutamate receptors. Disruption of this signaling system has been implicated in synaptic dysfunction and cognitive decline associated with Alzheimer's disease (AD). In this study, we investigated the immunodensities of neuronal pentraxin 1 (NPTX1), neuronal pentraxin 2 (NPTX2), and their receptor (NPTXR) in the dorsolateral prefrontal cortex (dlPFC) of a large post-mortem cohort (n = 219) from the Rush Memory and Aging Project (RMAP), integrating ante-mortem cognitive assessments with detailed neuropathological evaluations.
Protein levels were quantified by Western blot in crude dlPFC homogenates using target-selective antibodies. For NPTXR, both full-length (FL) receptor and its N-terminal and C-terminal fragments were measured to assess receptor processing and fragmentation. Linear regression models adjusted for age, sex, and years of education were applied to examine associations between protein immunodensities, global cognitive performance proximate to death, and AD-related neuropathology, including tau tangle burden.
Our analyses revealed that higher levels of NPTX2 and both NPTXR fragments were significantly associated with better cognitive performance and lower tau pathology. In contrast, NPTX1 and full-length NPTXR showed no significant relationships with either cognitive or neuropathological measures. Stratification by Braak stage demonstrated that NPTX2 remained a robust predictor of cognitive status across all stages of neurofibrillary degeneration. Conversely, associations between NPTXR fragments and cognition were restricted to early Braak stages, suggesting a stage-dependent role of receptor fragmentation in disease progression.
These findings support a model in which preserved NPTX2 expression and regulated processing of NPTXR are linked to cognitive resilience and reduced AD pathology. The differential associations observed between full-length and fragmented NPTXR further suggest that receptor cleavage may reflect or modulate synaptic adaptations during early disease stages. Alterations in the neuronal pentraxin system, particularly involving NPTX2 and NPTXR fragmentation, are closely linked to cognitive function and tau pathology, highlighting their potential role as synaptic biomarkers and modulators in Alzheimer's disease progression.

Authors
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Reference number: T01-2

ALTERATIONS IN CALCIUM HOMEOSTASIS AND ENDOPLASMIC RETICULUM-MITOCHONDRIA CONTACTS IN ACTIVATED MICROGLIA IN EXPERIMENTAL MODELS OF PARKINSON´S DISEASE.

Parkinson's disease is characterized by neuronal degeneration in substancia nigra and also by neuroinflammation, where microglia plays a central role. Increasing evidence suggests that α-synuclein aggregation contributes to microglial dysfunction, although the cellular mechanisms involved remain incompletely understood. Moreover, intracellular calcium signaling and endoplasmic reticulum (ER)–mitochondria communication have emerged as relevant processes in neurodegeneration.
The aim of this study was to investigate whether α-synuclein preformed fibrils (PFFs) modulate calcium homeostasis and ER–mitochondria interactions in human induced pluripotent stem cell (iPSC)-derived microglia under inflammatory conditions. Cells were treated with LPS, IFNγ, alone or in combination with PFFs. Intracellular calcium dynamics were measured by calcium imaging, and ER–mitochondria contacts were evaluated by Proximity Ligation Assay (PLA) using VDAC and IP3R3 antibodies.
Preliminary results indicate that LPS and IFNγ stimulation enhances intracellular calcium responses in iPSC-derived microglia. Consistently, those treatments also increased VDAC–IP3R3 PLA signal, suggesting enhanced ER–mitochondria interaction under inflammatory conditions. In contrast, exposure to α-synuclein PFFs appeared to attenuate these effects, supporting the idea that α-synuclein may interfere with ER–mitochondria communication and calcium signaling in activated microglia. Interestingly, IFNγ combined with PFFs seemed to recover the ER-mitochondria in the PLA assays, suggesting a neuroprotector mechanism.
Overall, these findings imply that inflammatory activation promotes functional ER–mitochondria coupling in human microglia, whereas α-synuclein aggregation may disrupt this response. This mechanism could contribute to microglial dysfunction in experimental models of Parkinson's disease.

Authors

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Reference number: T01-3

GENERATION AND ISOLATION OF HIPSC-DERIVED OLIGODENDROCYTES TO IDENTIFY DIFFERENTIALLY EXPRESSED OLIGODENDROGLIA MARKERS IN APOE3 AND APOE4 GENOTYPES.

Alzheimer's disease is the most prevalent neurodegenerative disorder, characterized by dementia and irreversible memory loss. In addition to age, the main risk factor is the presence of specific genetic variants, with the E4 allelic variant of the APOE gene being the one most strongly associated with the disease: carrying one copy increases the risk of developing Alzheimer's disease three- to fourfold, whereas carrying two copies increases the risk eight- to twelvefold. Evidence indicates that APOE4 significantly alters signaling pathways involved in cholesterol biosynthesis, homeostasis, and transport in mature oligodendrocytes, directly interfering with the myelination process. However, not all oligodendroglia-specific markers whose expression is altered by the presence of

the APOE4 allele have yet been identified. In the present project we aim to identify oligodendroglia markers that are differentially expressed in APOE3 and APOE4 genotypes, to identify new molecular targets in non-myelinating early oligodendrocytes that may be used in the development of therapies to prevent the demyelination that occurs in the disease. For that, we have differentiated human oligodendrocytes from induced pluripotent stem cells (iPSCs) of both genotypes. Next, we have used fluorescence-activated cell sorting (FACS) to isolate O4+ oligodendrocytes, of whose RNA expression we will analyze by bulk RNA sequencing in the near future.
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Reference number: T01-4

CANNABINOID CB1 REGULATION OF LONG-CHAIN SPHINGOLIPIDS IN MYELIN ENGAGES S1P1 RECEPTOR SIGNALING IN A RAT MODEL OF ALZHEIMER-TYPE DEMENTIA

In dementia of the Alzheimer's type (DAT), impairments in cognition are associated with alterations in the cholinergic system as well as myelin disturbances in brain regions involved in memory processing. Cannabinoid-based interventions are capable of influencing these changes in a DAT rat model generated through selective cholinergic neuron lesions (192IgG-SAP) in the nucleus basalis magnocellularis (NBM). Endocannabinoids (eCBs), which originate from membrane lipids, stimulate CB1 receptor pathways and promote ceramide formation, the precursors of sphingosine-1-phosphate (S1P), primarily through S1P1 receptor signaling.
The present work investigated myelin-associated lipid species and S1P1 receptor activity following subchronic administration of WIN55,212-2 (0.5 mg/kg for 5 days) in this experimental rat model. Cognitive performance was evaluated with the passive avoidance (PA) task in four experimental conditions (aCSF, 192IgG-SAP, aCSF+W, and 192IgG-SAP+W). Myelin integrity was assessed by MBP immunostaining, S1P1 receptor function through [35S]GTPγS autoradiography, and lipid profiling using MALDI-MSI. Both lesion induction and cannabinoid treatment affected PA outcomes. In cortical layers I–II, MBP expression decreased in the 192IgG-SAP group, whereas WIN administration restored values comparable to controls. Within the corpus callosum, MBP levels were significantly higher in the 192IgG-SAP+W group compared with 192IgG-SAP alone. Lipidomic analyses revealed that WIN exposure modified several myelin-associated lipids, including sulfatides (such as ST(d18:1/18:0) and ST(d18:1/24:0)), phosphatidylethanolamines (e.g., PE 36:0+K+), and phosphatidylserines (e.g., PS(P-20:0/22:2)+Cl-). In white matter regions, increases were observed in long-chain ceramides (e.g., Cer 42:2;O2+H-H2O) and galactosylceramides (e.g., GalCer 42:2;O2+K+). S1P1 receptor activity was elevated in the globus pallidus and hippocampus of the aCSF+W group, while it declined in the corpus callosum. In animals treated with 192IgG-SAP+W, S1P1 signaling increased in the hippocampus but decreased in the amygdala. In contrast, lesioning the NBM almost completely suppressed S1P1 activity, and WIN

treatment did not reverse this effect. Additionally, the intensity of certain lipid species, including oleoylcarnitine and hexosylceramides, showed correlations with S1P1 receptor activity in the NBM.
Overall, WIN55,212-2 appeared to enhance remyelination by reshaping the lipid composition of myelin, in parallel with alterations in S1P-related signaling pathways. These results indicate that cannabinoid-driven regulation of sphingolipid metabolism could represent a promising therapeutic strategy for neurodegenerative disorders characterized by demyelination.
Keywords: Sphingolipid, Cholinergic system, CB1 receptor, S1P.
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Reference number: T01-5

ASTROCYTE VULNERABILITY TO PATHOLOGICAL TAU IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, synaptic dysfunction, and accumulation of misfolded protein aggregates, particularly amyloid-beta and tau. These pathological aggregates strongly impair neuronal function and stability, finally leading to neurodegeneration. Despite increasing evidence supporting the role of astrocytes in AD pathology, the effects of pathological tau aggregates on astrocyte homeostasis remain poorly understood.
In this study, we investigated how pathological tau aggregates affect astrocytic responses and behavior in primary astrocyte culture, while also analysing the distribution of tau aggregates across different cell populations in human AD tissue. Primary astrocytes were isolated from two different brain regions, cortex and midbrain, to evaluate potential region-specific differences in function and vulnerability. Astrocytes were chronically (5 days) treated with tau and a-synuclein in both monomeric and pre-formed fibril (PFF) species, either alone or in combination. Cellular impairments were then assessed using live-cell calcium imaging, immunofluorescence, and the tau-Proximity Ligation Assay (Tau-PLA), which detects early tau aggregates. In addition, post-mortem human brain tissue from control and AD patients of different Braak stages was analyzed using Tau-PLA and immunohistochemistry, allowing cell-type specific mapping of early tau aggregates in different brain regions.
Overall, chronic aggregate treatment altered astrocytic calcium response, increased the expression of reactive markers, and impaired lysosomal pathway function. In human AD tissue, we observed region-specific variations in the distribution of tau pathology. Across the selected regions, tau aggregates showed a uniform cell-type-dependent pattern, with the highest proportion detected in neurons, a lower but substantial fraction in astrocytes, and an even lower presence in microglia.

These findings suggest that astrocytes can internalize and process pathological tau, leading to the impairment of their biological processes and functions, thereby emphasizing their involvement in AD pathogenesis and their susceptibility to tau-related pathology.

Authors
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Reference number: T01-6

FINGOLIMOD AMELIORATES SCOPOLAMINE-INDUCED SPATIAL MEMORY DEFICITS THROUGH SPHINGOSINE-1-PHOSPHATE RECEPTOR MODULATION

Neurolipid receptors have been implicated in neuroprotective mechanisms that may counteract cognitive deficits. In this context, we assessed whether the modulation of sphingosine-1-phosphate signalling pathway using fingolimod (FTY720) could exert a neuroprotective effect. Specifically, we investigated whether biochemical modifications induced by fingolimod could reverse scopolamine-induced spatial memory loss. Spatial memory was examined in the Barnes Maze using Sprague Dawley rats treated with a daily intraperitoneal fingolimod (0.5, 1, 3 mg/kg) for 5 days and a single scopolamine dose (2 mg/kg) before the fifth day probe. Acquisition trials measured speed, latency, and distance, whereas the probe trial measured time spent in each quadrant. The collected samples were analyzed by Western blot (WB) to determine S1P1 receptor expression levels in the cortex and hippocampus.
The scopolamine treated group (SCOP) spent less time in the target quadrant compared to vehicle group (VEH) (51.03±3.21 s, n=14 vs 88.64±6.79 s, n=12, ****p≤0.0001), confirming memory impairment. This deficit was reversed by fingolimod at 3 mg/kg (FTY3mg/kg) (82.02±6.20 s, n=12, ***p≤0.001). Unlike SCOP animals, fingolimod-treated groups showed a preference for the target quadrant, with SCOP+FTY3mg/kg displaying the strongest effect (p≤0.0001). This cognitive improvement is expected to be closely correlated with neurochemical alterations in S1P1 receptor expression observed by Western blot analysis.
In conclusion, fingolimod mitigated scopolamine-induced spatial memory deficits through modulation of the sphingosine-1-phosphate pathway. In this way, fingolimod acts as a palliative mechanism, counterbalancing the functional impairment caused by scopolamine.

Authors
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Reference number: T01-7

LPS AND IFNΓ ELICIT DISTINCT CELLULAR FUNCTIONS IN A TRI-CULTURE OF MURINE ASTROCYTES AND HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS AND MICROGLIA

Parkinson's disease (PD) is a chronic, progressive, and complex neurodegenerative disorder characterized by the accumulation of α-synuclein (α-Syn) and glial dysregulation. To better understand the interplay between them, we generated induced pluripotent stem cells (iPSCs) derived dopaminergic neurons and microglia, and co-cultured them with murine astrocytes producing an advanced tri-culture model for PD. To investigate the interaction between aggregation, microglial response, and neuronal health/survival this tri-culture was treated with: α-syn pre-formed fibrils (PFFs), together with Lipopolysaccharide (LPS) or interferon γ (IFNγ).

We found that PFF and LPS reduced the stained area by tyrosine hydroxylase (TH) while IFNγ treatment provided a significant rescue of TH levels back to control levels. This was also reflected in an increase in the synaptic apposition area in IFNγ treated cells, indicating an improvement in the established connections between neurons. Microglia showed an expected increase in the number of cells after IFNγ treatment concomitant with a restoration of control lysosomal counts per cell. To understand whether the observed effects on the tri-culture were due to the response of microglia to treatments, we decided to investigate a monoculture of microglia treated acutely and chronically with PFFs and LPS/ IFNγ.

When treated with PFFs, acute and chronic IFNγ treatment led to a decrease in lysosomal area and the a-syn area within lysosomes, indicating increased a-syn degradation. However, acute and chronic LPS treatment produced a dramatic decrease in phagocytic capacity in both vehicle and PFF treated cells, which was accompanied by an increase in the lysosomal area of the cells comparable to that of PFF-treated microglia, and an increase in the a-syn area within lysosomes, indicating decreased a-syn degradation.

Overall, all these results indicate that LPS and IFNγ affect microglial function distinctly, and that glial cell communication plays a crucial role in neuronal survival.

Authors

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Reference number: T01-8

BACLOFEN TREATMENT PROMOTES OLIGODENDROCYTE DIFFERENTIATION AND MITIGATES ANXIETY-LIKE BEHAVIOR AND SYSTEMIC INFLAMMATION IN MOUSE MODELS OF ALZHEIMER´S DISEASE

Alzheimer's disease (AD) is increasingly recognized not only as a neuronal pathology but also as a condition involving significant myelin disruption. Oligodendrocytes (OLs), the myelinating cells of the CNS, are vital for neuronal health and are affected early in AD progression. Our group has previously identified GABAB receptors (GABABRs) as key modulators of the OL lineage, promoting OPC survival and maturation both in vitro and

in vivo. In this study, we evaluated the therapeutic potential of the GABABR agonist baclofen (Bac) in two complementary AD models.
To this end, 15-month-old (15M) and 12M 3xTg-AD mice, alongside 4M 5xFAD mice, received daily intraperitoneal injections of saline or baclofen for 30 days. Behavioral testing was conducted during the final week of treatment, followed by plasma collection and immunohistochemical analysis of the OL lineage at the end of the 30-day period.
Our histological analysis in 15M 3xTg-AD mice revealed region-specific OL dynamics: while the paramedial corpus callosum (CC) remained stable, the lateral CC exhibited a significant reduction in OLs, compared to wild-type (WT) mice, regardless of treatment. Notably, in the dentate gyrus (DG), 3xTg-AD mice displayed an imbalanced OL population with respect to WT, characterized by an accumulation of OPCs and immature OLs together with a loss of mature OLs, which was restored successfully by baclofen treatment. This cellular recovery was accompanied by behavioral improvements in 12M 3xTg-AD mice, where baclofen exerted a potent anxiolytic effect in the Open Field (OF) and Elevated Plus Maze (EPM) tests, correlating with a reduction in plasma corticosterone levels.
In the more aggressive 4M 5xFAD model, mice displayed a phenotype of hyperlocomotion and pathological disinhibition in the EPM test, as well as reduced spontaneous alternation in the Y-maze. Interestingly, while the impact of baclofen on these specific behavioral deficits remained limited, it demonstrated a robust efficacy on the systemic environment. Specifically, baclofen led to a significant reduction in the plasma levels of the pro-inflammatory cytokines TNF-α, IL-1β, IFN-γ, IL-17A, and the chemokine MCP-1, an immunomodulatory effect observed exclusively in males across both genotypes.
Together, these findings highlight that GABABR activation by baclofen provides a multitarget therapeutic approach, promoting OL maturation while simultaneously mitigating anxiety and exerting a robust systemic anti-inflammatory effect.
This research was funded by Basque Government (2025333045, PIBA-24-0037, IT1551-22) MICINN (PID2022-140236OB-I00), and MICINN grant to BI.OB (FPU20/06365).
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Reference number: T01-9

FROM PRODROMAL STAGES TO NEURODEGENERATION: CHARACTERIZATION OF A PROGRESSIVE SYSTEMIC MODEL OF PARKINSON'S DISEASE

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuron loss and α-synuclein aggregation, involving both motor and non-motor symptoms. Non-motor manifestations such as olfactory dysfunction, constipation, and sleep disorders may precede motor impairment and remain poorly represented in current animal models. This study characterizes a novel murine PD model based on systemic administration of the AAV9-P31-hTyr viral vector, designed to reproduce the slow progression of the disease, enabling the study of prodromal symptoms, including early-stage dysfunctions and the gut-brain axis. In a second

approach, the model will be used to investigate the therapeutic effect of PLA2G4E after disease onset, as a novel strategy for PD treatment.
SNCAWT and FVB/NJ mice of both sexes received retro-orbital injections of AAV9-P31-hTyr, while controls remained untreated. Behavioral studies showed that FVB/NJ mice developed significant olfactory impairment three months after vector administration, supporting the hypothesis that peripheral non-motor symptoms appear before motor deficits. In contrast, SNCAWT mice displayed reduced exploratory activity, lower locomotor performance, and anxiety-like behavior, limiting olfactory assessment and suggesting intrinsic differences related to α-synuclein expression.
Overall, the PIGMO model represents a promising platform for investigating progressive PD pathology and improving the understanding of early disease mechanisms associated with both motor and non-motor symptoms.

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Reference number: T01-10

P2X4 DRIVES SEX-SPECIFIC NEUROPROTECTION IN AUTOIMMUNE NEUROINFLAMMATION

Microglia contribute to multiple sclerosis (MS) pathophysiology through debris clearance, remyelination, and regulation of neuroinflammation. These processes are partly mediated by the ATP-gated ion channel P2X4, predominantly expressed in microglia. We previously reported that ivermectin (IVM), a positive allosteric modulator of P2X4, influences microglial activation, enhances myelin phagocytosis, and improves functional recovery in experimental autoimmune encephalomyelitis (EAE). To define accurately the selectivity of the pharmacological treatment and the role of microglia in the protective effect, we used P2X4mCherryIN knock-in (P2X4KI) mice, in which P2X4 displays increased surface localization due to reduced internalization. ATP-evoked currents were enhanced in P2X4KI microglia, and transcriptomic analyses indicated reduced inflammatory and immune-related pathways. Both constitutive and myeloid-specific P2X4KI mice showed an amelioration of motor symptoms at all EAE stages in female mice, but not in males. Ovariectomy abolished this benefit, suggesting a role for female hormones. Consistently, progesterone potentiated P2X4 currents and modified channel kinetics. Overall, these findings support a role for P2X4 in shaping microglial responses during neuroinflammation and suggest that hormonal context may influence its functional impact.

Authors
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Reference number: T01-11

DISTRIBUTION AND ACTIVITY OF S1P1 RECEPTORS IN THE BRAIN OF HUMAN AND RODENT MODELS

Sphingosine 1-phosphate (S1P) is an important lipid neurotransmitter and neuromodulator in the central nervous system (CNS). Among its receptors, S1P1 is the most abundant and functionally active GPCR in the brain, with established roles in neuroprotection, including anti-apoptotic, proliferative, and cell-trafficking processes. Characterizing the distribution and functional activity of S1P1 receptors is therefore essential for the development of therapies targeting neurodegenerative diseases.
In this study, S1P1 receptor distribution in murine and human brain tissues was analyzed by autoradiography using the novel selective radioligand [3H]CS1P1. Tissue sections were incubated with 5 nM [3H]CS1P1, while non-specific binding was determined with the selective S1P1 antagonist NIBR-0213. Receptor functionality was further assessed by [35S]GTPγS autoradiography in the presence of the S1P1 agonist CYM5442, enabling comparisons between receptor density and signaling activity across brain regions.
Results revealed widespread S1P1 expression throughout the CNS, with markedly higher receptor density in grey matter than in white matter regions such as the corpus callosum. Although receptor density generally correlated with functional activity, this relationship differed across brain regions and species. For example, increased density was associated with enhanced activity in the cingulate cortex, whereas inverse patterns were observed in the rat hippocampal dentate gyrus.
Overall, these findings highlight the complex and region-specific role of S1P1 receptors in the CNS and support their potential as therapeutic targets for neurodegenerative disorders, including Alzheimer's disease.

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Reference number: T01-12

INVESTIGATING THE INTERPLAY OF INTERHEMISPHERIC CONNECTIONS, BILINGUALISM, READING, AND GENETICS

While language is typically left-lateralised, both bilingual individuals and individuals with dyslexia show reduced hemispheric lateralisation, and yet exhibit markedly different reading outcomes. We hypothesised that interhemispheric connectivity, particularly via the corpus callosum (CC), may underlie this divergence. Specifically, bilingualism may promote adaptive hemispheric cooperation, whereas in dyslexia it may reflect compensatory mechanisms to a left hemisphere dysfunction. Here, we explored the possibility of early bilingualism as a protective factor of dyslexia, acting through modifications in the CC structure.
To test this, we analysed MRI, genetic, and behavioural data from over 10,000 children in the Adolescent Brain Cognitive Development (ABCD) study. Mixed-effects and mediation models were used to examine the effects of a continuous bilingualism index and polygenic scores for reading difficulties on reading outcomes, with CC structure and overall brain size as mediators.

We found that bilingualism had specific positive effects on reading, partially mediated by the anterior CC, independently of the overall brain size. In contrast, polygenic scores of reading difficulties seemed to influence reading performance through brain size rather than CC. These findings suggest different neural pathways influencing reading development and support the strong potential of bilingual experience to strengthen spectrum-wide resilience to reading difficulties.

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Reference number: T01-13

IDENTIFICATION OF BIOLOGICAL PHENOTYPES IN A COHORT OF PEOPLE WITH MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic, immune-mediated demyelinating disease of the central nervous system characterized by inflammatory damage to the myelin sheath surrounding axons. Beyond its neurological manifestations, MS is increasingly recognized as a multifactorial disorder in which environmental and lifestyle-related factors may contribute to disease progression and symptom burden. In this context, growing evidence indicates that both the composition and function of the gut microbiota are altered in people with MS. Among the factors shaping the intestinal microbiota, diet and lifestyle have emerged as key modulators. At the same time, MS markedly affects patients' quality of life, with fatigue, anxiety, depression representing some of the most prevalent comorbidities.

Given the complex interplay between microbiota, lifestyle, inflammation, metabolism, and clinical manifestations, this study aimed to perform a comprehensive characterization of a cohort of people with MS from Gipuzkoa through a multidimensional integrative approach.

A total of 57 individuals with MS were included in this observational study. Data collection comprised gut microbiota profiling using 16S rRNA gene sequencing, short-chain fatty acid (SCFA) quantification, inflammatory marker assessment, lifestyle and dietary habit evaluation, quality-of-life measurements questionnaires, blood biochemistry analyses, and body composition measurements.

The analysis revealed a high prevalence of factors associated with impaired health status and reduced well-being. Biochemical analyses showed elevated cholesterol levels in a large proportion of participants. Quality-of-life assessments indicated that 47.4% of patients experienced severe fatigue, with physical fatigue representing the most affected dimension. Moreover, 52% of participants presented possible or probable anxiety, while 33% showed possible or probable depression. Body composition analysis further revealed that 20% of participants were classified as having obesity or severe obesity.

To better understand cohort heterogeneity, an integrative clustering analysis was conducted. This approach identified four distinct biological phenotypes. The largest clusters included the microbiota–QoL associated phenotype and the dysbiotic-behavioral risk phenotype. The third cluster was associated with metabolic-functional impairment, while the fourth represented an inflammatory risk phenotype. A microbiological characterization was performed for each cluster to describe their gut-

associated signatures, supporting the relevance of gut microbiota in the stratification of systemic disease phenotypes.
Overall, these findings highlight the marked heterogeneity of MS and support the use of multidimensional approaches to better understand host- microbiota interactions. The identification of distinct biological phenotypes may facilitate the development of personalized nutritional and therapeutic strategies aimed at improving patient well-being and disease management. In this context, targeted nutritional interventions could represent a promising approach to modulate metabolic and inflammatory status and enhance quality of life in people with MS.

Authors
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Reference number: T01-14

ANALYSIS OF THE EFFECTIVENESS OF A GROUP PSYCHOEDUCATIONAL INTERVENTION PROGRAMME FOR THE MANAGEMENT OF MIXED CHRONIC BACK PAIN (COCOAP-DOLOR PROJECT)

Chronic mixed back pain (neuropathic and nociceptive) affecting the cervical, thoracic or lumbar spine is a condition that affects the spine for a period of more than three months, with a prevalence of 17% in the general population. This condition has a multifaceted impact on quality of life, emotional well-being and cognitive function, and is associated with high levels of absenteeism and sick leave.
This study evaluates the effectiveness of the psychoeducational module of a Cognitive Behavioural Therapy (CBT) programme, delivered in a face-to-face, group setting, on the three areas mentioned above in patients with mixed-type DCE. In accordance with the objectives of the CoCoAp-Dolor project, approved by the CEIm-E (code: PS2022006), a quasi-experimental pre-post test design was applied to a convenience sample of 31 patients at Quirón Salud Bizkaia Hospital. The sample was divided into two randomly assigned groups: clinical (n = 16; mean age 52.72 ± 9.327) and control (n = 15; mean age 51.8 ± 8.661). The intervention consisted of 10 sessions (90 min) over three months. The following were assessed: a) Physical functioning, impact and pain level, sleep quality and coping strategies (Roland-Morris Questionnaire; WHYMPI; Numerical Rating Scale; PSQI; and CSQ); b) emotional state (HADS); and c) cognitive functioning in terms of memory, attention and executive functions (TAVEC; FCR; STROOP; SDMT; TMT A and B; and PMR-A).
The data were analysed using non-parametric tests (Mann-Whitney U and Wilcoxon). In the post-test phase, the experimental group showed better performance than the control group in immediate verbal memory ($p = .033$) and verbal fluency ($p = .028$); improved sleep quality ($p = .041$) and reduced pain intensity ($p = .028$). In the within-group analysis (pre-test–post-test), the experimental group showed a reduction in the impact of pain ($p = -.049$), physical disability ($p = .017$) and anxiety-depressive symptoms ($p = .013$); and an increase in executive function performance ($p = .035$) and inhibition control ($p = .012$). No relationship was observed between changes in cognitive functions and the levels of clinical variables. In contrast, the level of pain impact appears to be related to anxiety-depressive symptoms and sleep quality.

In conclusion, the results indicate that the psychoeducational component of CBT is effective in reducing anxiety and depressive symptoms, as well as the intensity and impact of pain. Changes in cognitive functioning do not appear to be attributable to reductions in levels of depression, anxiety, or the intensity and impact of pain, suggesting a dissociative effect.

Authors

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Reference number: T01-15

REDUCED CLASS II HDAC ACTIVITY IN PREFRONTAL CORTEX OF SCHIZOPHRENIA AND BIPOLAR DISORDER SUBJECTS: POSSIBLE EVIDENCE FOR INHIBITION BY ANTIPSYCHOTIC DRUGS

Schizophrenia and bipolar disorder share etiological factors and show overlapping symptomatology (1). Moreover, treatment of schizophrenia subjects is based on antipsychotic drugs, which are also first-line drugs in bipolar disorder due to their antimanic and mood-stabilizing properties. Susceptibility to develop schizophrenia and bipolar disorder is determined by interactions between genes and environment, possibly via epigenetic mechanisms. Histone deacetylases (HDAC) are key epigenetic enzymes in the regulation of gene expression and have been postulated as possible targets of antipsychotic and mood stabilizing drugs (2). Studies in our laboratory have shown that class I HDAC activity is reduced in dorsolateral prefrontal cortex (DLPFC) of schizophrenia subjects treated with antipsychotics (3) but not in bipolar disorder subjects. However, little is known about class II HDAC expression in brains of subjects with schizophrenia and bipolar disorder (4,5) and kinetic characterization of class II HDAC in these disorders is still missing. Thus, the aim of this study was to evaluate class II HDAC activity in the DLPFC of schizophrenia and bipolar disorder subjects.

To this end, DLPFC samples of two cohorts formed by cases of schizophrenia (n=19) and bipolar disorder (n=18) were paired to sex-, age- and postmortem delay-matched control subjects. Both schizophrenia and bipolar disorder subjects were classified in antipsychotic-treated or antipsychotic-free subgroups according to blood toxicology at the time of death. Class II HDAC activity on fluorogenic Boc-Lys(TFA)-AMC substrate was evaluated in nucleosolic DLPFC fractions. Data were resolved via Michaelis-Menten non-linear regression and kinetic parameters of maximum velocity (Vmax) and Michaelis constant (KM) were obtained. For both cohorts, statistical comparison of data fitting to a global or to 2 separate curves in cases and controls was done using an extra sum-of-squares F test. Complementary experiments explored possible differences in the protein density of class II HDAC enzymes HDAC4 and HDAC5.

Class II HDAC activity was not different from respective controls neither in schizophrenia nor in bipolar disorder cohorts. Nevertheless, differences were observed in the subjects with positive toxicology for antipsychotics. Antipsychotic-treated schizophrenia subjects showed a slight trend towards a lower activity compared to respective controls ($F_{2,80} = 1.51$, $p = 0.226$) due to differences in Vmax (-26%). This tendency reached statistical significance in antipsychotic-treated subjects of the bipolar disorder cohort ($F_{2,79} = 17.71$, $p<0.0001$; Δ in Vmax = - 68%). By contrast, HDAC4 and HDAC5 protein density remained unaltered in all comparisons.

Altogether, past and present results on class I and II HDAC activity suggest that the mechanism of action of antipsychotic drugs may involve selectively reducing HDAC activity in brains of subjects with either schizophrenia or bipolar disorder. The implications of the differential epigenetic regulation by antipsychotic drugs in these disorders deserves further study.

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Reference number: T01-16

BEHAVIOURAL AND NEUROCHEMICAL EFFECTS OF PSILOCYBIN FOLLOWING CHRONIC STRESS EXPOSURE IN MICE

Major depressive disorder (MDD) is a common psychiatric condition with limited treatment efficacy, driving interest in novel therapies such as psilocybin. However, its neurobiological mechanisms remain unclear. Chronic stress, a key risk factor for MDD, is widely used in translational animal models of depression. The present study aimed to investigate the effects of the psychedelic 5-HT2A receptor agonist psilocybin in a chronic stress animal model by assessing physiological stress-related alterations, psychedelic-like behavioural responses, and cortical monoamine levels. Male and female C57BL/6J mice were randomly assigned to four experimental groups: control (n=24), psilocybin (n=24), chronic multimodal stress (CMMS; n=24), and CMMS + psilocybin (n=24). The CMMS protocol was conducted over 21 days and consisted of social isolation, movement restraint in ventilated Falcon tubes (twice a day, 2 h/session), and alternating nocturnal stressors (untoned radio or stroboscopic light). Psilocybin (5 mg/kg, i.p.) or saline was administered on day 14 of the protocol. Physiological assessments included body and adrenal gland weights, whereas psychedelic-like behavioural activity was evaluated using the head twitch response (HTR). Cortical monoamine concentrations were quantified by high-performance liquid chromatography (HPLC). Data were analysed using three-way ANOVA followed by Tukey post hoc tests after statistical significance. Chronic stress exposure induced marked physiological alterations, including reduced body weight for

both, male (F[1,44]=63.66, p<0.0001) and female (F[1,44]=19.28, p<0.0001) mice along time and increased adrenal gland weight (F[1,86]=51.42, p<0.0001). HTR analysis revealed a robust effect of psilocybin administration in both sexes (F[1,87]= 90.67, p<0.0001). Significant stress × psilocybin interactions were also observed (F[1,87]= 27.16, p<0.0001), indicating that chronic stress decreases the behavioural response to psilocybin. Regarding neurochemical measures, stress exposure significantly increased cortical noradrenaline (NA) levels regardless of sex (F[1,85]= 13.01; p=0.005), while dopamine (DA) and serotonin (5-HT) concentrations remained unchanged across experimental conditions. Psilocybin administration did not significantly modulate cortical monoamine levels nor interact with stress exposure in either sex. Thus, chronic stress exposure induced significant physiological and neurochemical alterations, supporting the translational validity of the CMMS paradigm as a model of stress-related depressive phenotypes. Psilocybin elicited robust psychedelic-like behavioural responses, as reflected by increased HTR, without significantly modifying cortical 5-HT concentrations. Importantly, the observed stress-dependent modulation of HTR suggests that chronic stress may alter sensitivity to the acute behavioural effects of psilocybin.

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Reference number: T01-17

MODULATION OF MITOCHONDRIAL FUNCTION BY PSILOCYBIN IN THE MOUSE HIPPOCAMPUS

Depression is one of the most prevalent mental disorders worldwide, and current pharmacological treatments remain ineffective in a substantial proportion of patients. Increasing evidence suggests that mitochondrial dysfunction may play a key role in the pathophysiology of depression. In parallel, there has been renewed interest in the therapeutic potential of psychedelic compounds, particularly due to their rapid and sustained antidepressant effects. Psilocybin, a naturally occurring psychedelic alkaloid, has shown promising clinical outcomes in disorders such as depression and anxiety. However, the biological mechanisms underlying these effects remain incompletely understood. This study aimed to investigate the effects of psilocybin on mitochondrial function and to explore its potential contribution to the drug's antidepressant-like effects in a chronic multimodal stress (CMMS) mouse model. Male and female C57BL/6J mice were randomly assigned to four experimental groups: control (n=24), psilocybin (n=24), CMMS (n=24), and CMMS + psilocybin (n=24). The chronic multimodal stress (CMMS) protocol was conducted over a 21-day period. CMMS protocol consisted in social

isolation, movement restraint in ventilated falcon tubes (2 times a day, 2h each) and an alternating night stressor (untoned radio or stroboscopic light). Psilocybin (5 mg/kg, intraperitoneal) or saline was administered on day 14 of the protocol. Animals were euthanized on day 22, and hippocampal tissue was rapidly harvested for subsequent mitochondrial membrane isolation and functional analysis. We then studied the basal, maximal and net activities of complexes I, II and IV, GAPDH and MAO, by measuring the absorbance resulting from an enzymatic reaction using a spectrophotometer. All results were analyzed using two-way ANOVA, with multiple comparisons. Psilocybin treatment selectively increased basal activity of mitochondrial complex I and monoamine oxidase (MAO) in both male and female mice across control and CMMS conditions when compared to saline-treated groups (complex I: males $F[1,43] = 16.18$, $p < 0.001$; females $F[1,43] = 5.165$, $p < 0.05$; MAO: males $F[1,41] = 14.13$, $p < 0.001$; females $F[1,37] = 8.591$, $p < 0.01$, respectively). No significant main effects of stress exposure or stress × treatment interactions were detected, indicating that the CMMS protocol did not significantly alter mitochondrial activity under the present experimental conditions. Likewise, no significant changes were observed in the activity of mitochondrial complexes II and IV or in GAPDH activity in either sex. The increase in basal mitochondrial complex I and monoamine oxidase (MAO) activity in the hippocampus following psilocybin administration suggests an upregulation of bioenergetic function that may support the metabolic demands associated with rapid neuroplastic adaptations induced by the drug.

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Reference number: T01-18

THE SHAPE OF TRAUMA: HOW EARLY ABUSE AND NEGLECT INFLUENCES ANOREXIA NERVOSA

Anorexia nervosa (AN) is a severe and multifactorial psychiatric disorder in which childhood maltreatment and early traumatic experiences have been identified as key factors contributing to increased clinical severity and poorer prognosis. Evidence suggests that early adverse experiences may disrupt emotional development, cognitive functioning, and neurobiological processes, thereby increasing vulnerability to the onset and maintenance of AN.

The present study aims to examine the relationship between childhood trauma and anorexia nervosa, focusing on clinical symptomatology, environmental risk factors, and cognitive functioning.

A cross-sectional case-control design was employed, including female patients with AN and healthy controls recruited from clinical and general population. Multiple variables were assessed using standardized instruments. Sociodemographic variables include age,

educational level, and socioeconomic status. Childhood trauma and environmental adversity were measured using the CTQ-SF, TQ, and FES. Clinical variables include anxiety (STAI), depression (HDRS), self-esteem (Rosenberg Self-Esteem Scale), eating disorder symptomatology (EAT, EDI-3), body image disturbance (Gardner Body Image Assessment Scale), and global functioning (FAST, GAF). Neuropsychological functioning was evaluated using WISC-IV/WAIS-IV, WCST, IGT, TMT A/B, and MSCEIT. Data were analyzed using SPSS 27.0.

Compared with controls, patients with AN reported significantly higher rates of emotional (21% vs. 13.3%), physical (6.5% vs. 0%), and sexual abuse (19.7% vs. 6.7%) ($p < 0.05$), along with lower family cohesion. They exhibited elevated anxiety, depression, and eating disorder psychopathology, more pronounced body image distortion, reduced global functioning, and lower self-esteem ($p < 0.05$). Neurocognitive assessment revealed poorer performance in verbal reasoning and emotional processing tasks (WAIS-IV, MSCEIT).

By integrating environmental, clinical, and neurocognitive dimensions, this study aims to advance current etiological models of AN and to elucidate the mechanisms linking early adversity to disorder expression. A better understanding of these pathways may contribute to the development of more targeted, trauma-informed interventions, thereby improving clinical management and long-term outcomes.

Authors

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Reference number: T01-19

MAPPING LIPIDOMIC CHANGES AS SURROGATE BIOMARKERS OF STROKE PROGRESSION

Stroke triggers dynamic, time-dependent changes in brain lipid composition, which are crucial for neuronal survival and inflammatory responses. Characterizing these dynamics is challenging due to spatial and temporal heterogeneity. Here, we combine ultra-high-performance liquid chromatography (UHPLC) and matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) to achieve high-resolution, region-specific analysis of post-stroke lipid alterations.

Adult male Sprague-Dawley rats were subjected to transient middle cerebral artery occlusion (tMCAO, 75 min). Brains were collected at 1, 3, 7, 14, 21, and 28 days post-stroke, cryosectioned for MALDI-IMS or processed for lipid extraction and UHPLC analysis. MALDI-IMS mapped lipid spatial distribution at 100 µm/pixel, while UHPLC provided quantitative lipid identification. Adjacent sections underwent immunohistochemistry with anti-CD11b and anti-NeuN to assess neuroinflammation and neuronal loss.

Post-stroke lipid profiles exhibited dynamic, phase-dependent changes. PCA scatter plots revealed clustering by timepoint. In the acute phase (day 1), structural phospholipids, including phosphatidylethanolamine (PE) and phosphatidylinositol (PI), were reduced, reflecting acute neuronal injury and initiation of cell death. The subacute phase (days 3-7) was marked by accumulation of ceramides, cholesterol, and di- and tri-acylglycerols (DG and TG), consistent with peak inflammation and microglial activation. From day 14 onward, lipid profiles partially stabilized, suggesting attenuation of inflammation and tissue remodelling.

This integrated MALDI-IMS and UHPLC approach provides high-resolution spatiotemporal maps of lipid alterations after stroke, revealing temporal lipid signatures that track neuronal injury, inflammation, and tissue repair, and highlighting potential biomarkers and therapeutic targets for stroke recovery.

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Reference number: T01-20

IMAGING THE TEMPORAL DYNAMICS OF BLOOD-BRAIN BARRIER DYSFUNCTION AND EXTRACELLULAR MATRIX METALLOPROTEINASE INVOLVEMENT AFTER ISCHEMIC STROKE IN RATS.

Ischemic stroke is a cerebrovascular disease caused by interruption of blood flow to the brain triggering an ischemic cascade that disrupts the blood-brain barrier (BBB). During ischemia, increased BBB permeability allows the entry of proinflammatory mediators, contributing to brain damage. However, the mechanisms underlying BBB dysfunction and the role of matrix metalloproteinases (MMPs) during subacute and chronic phases after stroke remain poorly understood. The aim of this study is to evaluate BBB integrity, neuroinflammation and MMP activity during the first month after ischemia. Adult male Sprague Dawley rats (N=102) where subjected to a transient middle cerebral artery occlusion (tMCAO) for 75 min followed by reperfusion, and were studied at days 0, 1, 3, 7, 14, 21 and 28 post-stroke. In vivo BBB integrity was assessed using dynamic contrast-enhanced MRI (DCE-MRI), while ex vivo BBB permeability and vascular inflammation were measured by Evans Blue extravasation and VCAM immunohistochemistry. MMPs activity was quantified using [18F]BR351 PET imaging, as well as in situ and gel zymography. Our results showed BBB permeability increase during the first week, with a peak at day 7, followed by a recovery during the chronic phase. VCAM expression correlated with BBB leakage, indicating an association between vascular inflammation and BBB disruption. In contrast, [18F]BR351 signal and MMP-9 activity peaked within the first 24 hours, while MMP-2 activity peaked at day 7, likely contributing to vascular repair and remodeling. These findings suggest that subacute BBB disruption after subacute ischemic stroke is primarily mediated by inflammation rather than by MMPs activity.

Authors

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Reference number: T01-21

AUTOPHAGY-DEPENDENT MECHANISMS IN OLIGODENDROCYTE MATURATION AND WHITE MATTER INTEGRITY.

Autophagy is a key cellular mechanism supporting adaptation to the metabolic and structural demands associated with oligodendrocyte maturation and white matter maintenance. However, how alterations in autophagy-lysosome pathways impact oligodendrocyte physiology remains incompletely understood. Here, we investigated the role of autophagy in oligodendrocyte maturation using complementary in vivo genetic and in vitro pharmacological approaches.

In vivo, mice with constitutively reduced autophagic flux due to ATG4B deficiency exhibited reduced numbers of OLIG2 cells and a tendency towards decreased APC-CC1 oligodendrocytes specifically in the corpus callosum, with no detectable changes in the adjacent cortex, indicating that intact autophagic activity is required to sustain the oligodendrocyte lineage in white matter. Ongoing analyses aim to further define the affected maturation stage by examining progenitor populations and myelin-related markers.
In vitro, oligodendrocyte differentiation was associated with a consistent increase in autophagic flux together with an expansion of SCARB2 cytoplasmic compartments, suggesting enhanced engagement of autophagy-lysosome pathways during maturation. Pharmacological modulation of these pathways altered the organization of SCARB2 compartments. Functionally, inhibition of autophagy with MRT68921 or inhibition of SCARB2 with tetrandrine impaired oligodendroglial differentiation and reduced autophagic flux, whereas induction of autophagy with metformin or inhibition of glucocerebrosidase with conduritol b-epoxide did not affect autophagic activity or maturation, indicating selective requirements within the autophagy-lysosome system. Together, these findings support the idea that oligodendrocyte maturation and physiology depend on a finely regulated and selective organization of the autophagy-lysosome compartment, providing a framework to understand how oligodendrocyte homeostasis contributes to white matter organization.

Authors

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6.2 TOPIC 02: EXPERIMENTAL NEUROSCIENCE

Reference number: T02-1

PREFRONTAL CB1 RECEPTORS MODULATE EMOTIONAL AND COGNITIVE PROCESSES IN A SEX-DEPENDENT MANNER

The prefrontal cortex (PFC) integrates emotional and cognitive functions. CB1 receptors are widely expressed in this region, principally on GABAergic interneurons, and participate in the Excitation-Inhibition (E-I) balance. E-I alterations are described in psychiatric disorders, including depression, anxiety, and schizophrenia, where a reduction of CB1 has also been described. Both the endocannabinoid system and mood disorders display important sex differences.
In this work, we aim to elucidate the role of CB1 receptors in prefrontal GABAergic interneurons in the development of behavioural disorders in a sex-dependent way. For this objective, we deleted CB1 from 1) all PFC neurons, 2) the GABAergic population and 3) in the Parvalbumin-positive (PV) subpopulation of GABAergic neurons of adult CB1-flox male and female mice. PV neurons were also modulated using an optogenetic approach in a cohort of animals. One month later, mice were subjected to several behavioral tests to evaluate their emotional and cognitive performance. The GABAergic neuronal activity was studied via calcium imaging by fiber photometry.
Neuronal CB1 deletion in the PFC altered the innate emotional behavior in females, while GABAergic deletion affects mostly males. A phenotype aggravated in the (PV)CB1-KO animals.This effect correlated with lower activation of GABAergic neurons. Fear conditioning test revealed an impairment in learned-aversive responses in males and females. Immunofluorescence studies showed that global PFC CB1 deletion induced GABAergic-parvalbumin interneurons modulation with a sex-dimorphic pattern. Interestingly, the optogenetic modulation of these interneurons produced effects resembling those observed following CB1 deletion in PFC GABAergic neurons.
To summarise, PFC-GABAergic CB1 deletion induces an anxiogenic state and an exacerbated response to conditioned cues in a sex-dependent manner, potentially mediated by parvalbumin neuron activity. Thus, maladaptive prefrontal CB1 signalling could participate in the development of behavioral disorders.

Authors

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Reference number: T02-2

SEX-DEPENDENT INVOLVEMENT OF CB1 RECEPTORS IN INTERNEURONS DURING MEMORY PROCESSES

The endocannabinoid system (ECS) is a complex neuromodulatory system found in the brain. It is essential for maintaining brain homeostasis and is closely linked to the neurotransmitter release. Thus, it participates in regulating many brain functions, mainly through the type 1 cannabinoid receptor (CB1R), which acts as a neuromodulator across

distinct pathways in different brain structures, modulating neurotransmitter release and synaptic transmission, as well as regulating plasticity.
In this research, the goal has been to compare the role of CB1Rs in hippocampus-driven functions between male and female mice. The hippocampus is one of the most-studied brain structures and regulates many biological functions, including navigation and memory. The aim of this study is to investigate the influence of CB1Rs on memory. To do so, the de novo synthesis of CB1Rs was disrupted in DLX-positive interneurons of the CA1 area of both hemispheres' hippocampi, with the aim of investigating how the absence of these receptors influences behavior during different tests. In addition to these injections, an optical fiber was implanted in one of the hippocampi of each mouse to enable in vivo assessment of neuronal activity using the innovative Fiber Photometry (FP) technique during these tests. Thus, three behavioral tests were performed. On the one hand, for assessing innate emotional behavior, the Open Field (OF) test and the Elevated Plus Maze (EPM) were used. On the other hand, to analyze the mice's recognition memory, the Novel Object Recognition Test (NORT) was performed.
The results show that the deletion of CB1Rs in DLX-positive interneurons in the CA1 region of the hippocampus produced sex-dependent effects. In males, they showed the most significant effect on cognitive memory, indicating more negative results in the NORT test in the absence of these receptors, and, regarding anxiety, an anxiolytic effect prevailed. In the case of females, their greatest influence concerned emotional function and was necessary for the modulation of anxiety; the absence of the receptors makes the animal more vulnerable in more anxiogenic situations.

Authors
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Reference number: T02-3

EPILEPTIFORM ACTIVITY INDUCES SEX-DEPENDENT BEHAVIORAL CHANGES

Epileptiform activity (EA) refers to abnormal patterns of neuronal discharges detectable by electroencephalography. Although traditionally linked to epilepsy, EA is also present in aging and several neurological conditions, including autism spectrum disorder and Alzheimer's disease. Importantly, EA can occur without overt seizures, yet it has been associated with alterations in cognitive and emotional processing.
Despite its clinical relevance, the specific contribution of EA to cognitive dysfunction is still not fully understood, in part due to the lack of robust experimental models that isolate EA from other disease-related pathologies. To overcome this limitation, we employ an intra-hippocampal kainic acid (KA) model that induces persistent EA without eliciting seizures. Behavioral testing was conducted 50 days after induction to assess emotional and cognitive outcomes.
Our results indicate marked sex-dependent differences. EA females display deficits in cognitive performance, whereas KA-treated males do not show clear cognitive impairments but exhibit increased locomotor activity. To further link these behavioral effects to underlying neural mechanisms, we are conducting in vivo electrophysiological recordings in the hippocampus of freely moving mice. Preliminary observations suggest that EA selectively disrupts high-frequency oscillatory activity in females.

Authors

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Reference number: T02-4

SEX RELATED DIFFERENCES IN THE BRAINSTEM GLUTAMATE LEVELS AFTER ACUTE SOCIAL DEFEAT STRESS IN CD1 ADULT MICE

Exposition to acute social stress has shown to influence brain neurochemistry and bring physiological changes in both sexes. Despite that, there is a poor understanding in how stress responses differ between sexes. This study evaluated neurochemical differences of male and female CD1 adult mice after exposure to social defeat stress, targeting the brainstem as the region of interest. The neurochemical data was obtained through HPLC and analyzed with Jamovi. The results showcase an alteration in the glutamate pathway in relation to sex-stress, although no such effect was found in GABA. We found no significant interaction between sex and stress in catecholamine or tryptophan pathways. These findings suggest an alteration in the GABA-glutamate system due to sexually dimorphic responses to stress, underlining the need for further studies that help understand sex differences.

Authors

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Reference number: T02-5

PHENOTYPIC SIGNATURES OF CTNNB1 SYNDROME: LONGITUDINAL NEUROPSYCHOLOGICAL OUTCOMES

CTNNB1 syndrome is a rare neurodevelopmental disease resulting from pathogenic variants in the CTNNB1 gene. While its main clinical features have become increasingly well described, longitudinal information regarding cognitive, behavioural, and motor development remains scarce. The present study aims to provide longitudinal insights into the cognitive, clinical, and psychological characteristics of individuals with CTNNB1 syndrome. Data on cognitive, clinical, psychological, and neuropsychological functioning were collected at two separate time points. Longitudinal analyses revealed overall stability across most developmental domains, with no indications of systematic regression. Notably, a significant improvement in gross motor abilities was detected, particularly among younger participants. Linear mixed-effects modelling further indicated that age influenced developmental trajectories, as younger individuals showed greater progress over time in both gross motor skills and adaptive functioning compared with older participants. This study offers the first comprehensive longitudinal characterisation of CTNNB1 syndrome integrating neurodevelopmental follow-up. Overall, the findings suggest a pattern of gradual yet positive improvement in specific clinical domains.

Authors

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Reference number: T02-6

WHEN TIME SHAPES STRUCTURE: CELL CYCLE LENGTH DURING NEUROGENESIS IN AMNIOTES

Amniotes exhibit a remarkable diversity in brain size and morphology, raising fundamental questions about the developmental mechanisms underlying this variability. Among these, cell cycle dynamics in neural progenitor cells are a key factor linking neurodevelopmental processes with evolutionary outcomes. In particular, the duration of the cell cycle influences the total number of neurons generated, contributing to differences in brain size and organization across species.

Comparative studies suggest that cell cycle length varies among major amniote clades, with reptiles generally displaying longer cycles than birds and mammals, which exhibit faster proliferative dynamics. In addition to interspecific differences, cell cycle duration varies across development, with early stages showing shorter cycles and later stages a progressive lengthening that facilitates differentiation. However, available data remain limited to a few model species, and brain regions.

To address these gaps, we investigated cell cycle dynamics in three representative amniote species at early, intermediate, and late embryonic stages: mouse (Mus musculus), chicken (Gallus gallus), and gecko (Paroedura picta). Using a dual thymidine analogue labeling approach (EdU/BrdU) combined with Sox2 immunostaining to identify neural progenitors, we assessed cell cycle parameters based on the proportion of cells incorporating each marker, enabling quantitative comparisons of proliferative dynamics. This method allowed us to estimate cell cycle length and compare proliferative dynamics across developmental stages and eight brain regions from prosencephalon to rhombencephalon, including both alar and basal domains. This approach provides a framework to assess how spatiotemporal modulation of the cell cycle contributes to neurogenesis in amniotes. Our results expand current comparative datasets and shed light on the developmental mechanisms underlying evolutionary diversification of brain structure.

Authors
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Reference number: T02-7

DEVELOPMENTAL DIVERGENCES IN THE EVOLUTION OF THE TECTAL MESENCEPHALON OF AMNIOTES

Visual information is processed by homologous brain structures in all vertebrates. A primary brain center in the visual pathway is the tectal mesencephalon, also known as the superior colliculus in mammals or optic tectum in non-mammals. This brain structure has been interpreted as a conserved structure among species: not only it contains similar input and output projections with the rest of the brain in different species, but in all cases studied it is composed of different layers and the retinal information is arranged in a retinotopic manner. However, there are some clear differences between species: not only the relative size and architecture of the tectal mesencephalon and the lamina number is different between species, but the importance of this brain structure also changes between species, having a less important role in the visual information processing in mammals as the retinogeniculate

pathway gains more importance. How these differences take place at cellular level is unknown. In this research, we aim to study the possible conserved and differentiated features in the development of the tectal mesencephalon in Gallus gallus chickens, Paroedura picta geckos and Mus musculus mice that may explain how the divergencies between the optic tectum and the superior colliculus take place. To do so, we used EdU birthdating to mark the formation of tectal mesencephalon cells and layers, retrograde tracing techniques to explore the neuronal localization of the major axonal pathways arising from the optic tectum, and transcriptomics data analysis to explore the molecular features of the tectal mesencephalon of different amniote species. Preliminary data indicates that the neurogenic waves that form the tectal mesencephalon in amniotes seem to be very similar among these species. However, some connections seem to be non-existent in specific species. Most importantly, conserved connections between brain areas may arise in different developmental moments as the neurons giving rise to those connections are born in different neurogenic moments, showing that the developmental trajectories of the optic tectum and superior colliculus may not be completely conserved among species.

Authors
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Reference number: T02-8

HUMAN IPSC-DERIVED FOREBRAIN ORGANOIDS: A NOVEL MODEL TO INVESTIGATE CTNNB1 SYNDROME

CTNNB1 syndrome is a rare neurodevelopmental disorder with a broad spectrum of symptoms that primarily affects children worldwide, often misdiagnosed with cerebral palsy. It is caused by de novo loss-of-function mutations in the CTNNB1 gene, leading to dysfunction of β-catenin. Despite its impact, current knowledge about the disease remains limited, largely due to the absence of human-relevant models capable of recapitulating early brain development (Miroševič et al., 2025; Pallarès-Sastre et al., 2025).
Given the central role of β-catenin in cortical development, cell-cell adhesion, and Wnt-dependent transcriptional regulation, understanding how CTNNB1 mutations alter these processes is essential for uncovering the mechanisms driving the disorder. However, animal models fail to reproduce the complexity of human cortical architecture, and access to embryonic human tissue is extremely restricted, creating a critical gap in the field.
The generation of iPSC-derived forebrain organoids has enabled the development of the first human preclinical model for CTNNB1 syndrome. These 3D structures can recapitulate key aspects of early neurodevelopment (Lancaster et al., 2014) while preserving the patient's genetic background, making them a powerful tool for studying how specific CTNNB1 mutations, such as Tyr654* or Arg95*, affect neural lineage specification, tissue organization, and β-catenin function.
Our preliminary results indicate that CTNNB1-mutant organoids are more fragile, exhibit increased cell death, display fewer neurogenic niches, and show reduced nuclear β-catenin, all of which are consistent with impaired cortical development. Notably, we also observe substantial variability across different CTNNB1 mutant lines, suggesting

mutation-specific effects that may contribute to the heterogeneity of the disease phenotype.
These findings highlight the value of organoids as a significant advance in neurodevelopmental disorder research, providing a powerful platform to investigate how CTNNB1 mutations impair brain development and to support drug-screening efforts that may ultimately lead to targeted treatments and personalized medicine.

Authors
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Reference number: T02-9

TEMPORAL AND SPATIAL REGULATION OF MICROGLIAL TESSELLATION DURING DEVELOPMENT

During development, microglia, the resident immune cells in the brain, proliferate, and colonize the brain parenchyma until they reach a perfect tessellation covering all the brain parenchyma. This mosaic-like distribution is fundamental for the effective surveillance of the brain parenchyma, supporting precise immune responses of the microglial population. However, how this colonization is orchestrated to reach a tessellated distribution remains to be elucidated. To comprehend how microglia achieve their adult density and 3D tessellation we combined experimental data from postnatal mice and 3D mathematical modelling to define the contribution of potential spatial cues and test hypothesis such as contact-inhibition in the control of their proliferation and distribution. The analysis of the single nearest neighbour distance did not show significant differences across ages for all microglia, but proliferative microglia were more distant from each other compared to mitotically quiescent cells, suggesting a potential spatial cue in the control of microglial proliferation. To understand the colonization dynamics in space and integrate our experimental data, we developed a mathematical approach using a 3D agent-based model to analyze the local density of proliferative and mitotically quiescent microglia over time. We combined the model with experimental analysis of their spreading dynamics using long-term 3-photon in vivo imaging in P2-P4 mice, which allowed us to determine microglial velocity and trajectories during brain colonization. Elucidating the laws of attraction and repulsion controlling microglial tessellation will provide a better understanding of the microglial developmental trajectory towards functional maturity.

Authors
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Reference number: T02-10

CONSTRUCTION OF A DIENCEPHALIC CELL-TYPE ATLAS IN AMNIOTES THROUGH TRANSCRIPTOMIC DATA ANALYSIS

The diencephalon is the caudal part of the forebrain (or prosencephalon) that is located in the central region of the brain, above the brain stem. It processes a series of functions such as sensory and motor signal release or circadian rhythm regulation, among others.

The major structures of the diencephalon (the prethalamus, thalamus and pretectum) likely reveal a variety of evolutionary trends as diversification, conservation and innovation, making it highly interesting to study brain evolution. Understanding how its cellular diversity, connectivity, and developmental programs have evolved remains a major challenge in neuroscience. Thus, through the lens of evolutionary developmental biology (EvoDevo), this work aims to uncover the molecular profile of specific cell types in the amniote diencephalon to understand its cellular diversity. We analysed single-nuclei RNA sequencing data of the diencephalon of representative species of the major amniote non-mammalian clades (birds and reptiles) in order to build the first diencephalic transcriptomic atlas in these species. We tuned up the novel Fluent (Illumina) particle-templated instant partition sequencing protocol (PIP-seq) for chicken (Gallus gallus) and Madagascar ground gecko (Paroedura picta) neural tissue. We also analyzed mouse (Mus musculus) data through the information available in the literature. The intra- and inter-species comparison of the diencephalic transcriptomic cell-type profile will explain when and how lineage-specific innovations arise through amniote brain evolution. The preliminary large-scale transcriptomic atlas of different developmental stages for both species indicate a possible conserved molecular profile across major neuronal populations. Lastly, we seek to integrate this computational data with experimental neurogenesis and connectivity studies to bridge the gap between the chronological, functional and molecular constructions of cell-type identity that give rise to diencephalic novelties and species-specific complexity. Moreover, the proposed molecular analysis aims to provide a way to characterize neuronal populations resulting in cell-type classification frameworks for functional understanding.

Authors

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Reference number: T02-11

PPARΓ AS A CANDIDATE DOWNSTREAM EFFECTOR OF PLCB1-MEDIATED SIGNALING DURING NEURONAL DIFFERENTIATION OF NT2 CELLS

Previous studies identified nuclear PLCβ1 signaling as a critical regulator of neuronal differentiation in NT2 cells, where PLCβ1 overexpression is sufficient to induce neuronal phenotypes even in the absence of differentiation stimuli (1).In parallel, lipid signaling machinery involving PLCβ and DAGL activities has been detected within neuronal nuclei, suggesting a potential link between nuclear lipid signaling and transcriptional programs associated with neuronal fate acquisition (2,3). The integration of nuclear lipid signaling with transcriptional regulation during neuronal differentiation, however, remains poorly understood. Given its role as a lipid-sensitive transcription factor, PPARγ represents a potential downstream effector of PLCβ1-dependent signaling during neuronal differentiation.

Here, we investigated the relationship between PLCβ1 and PPARγ during AraC-induced neuronal differentiation of human NT2 cells, focusing on neuronal maturation and subcellular localization patterns.

Following AraC-induced differentiation, NT2 cells displayed marked morphological changes compatible with acquisition of a neuronal phenotype, accompanied by increased expression of the neuronal markers NF200 and NeuN. In parallel, PPARγ

expression progressively increased during neuronal differentiation and showed stronger immunoreactivity in cellular subpopulations exhibiting more developed neuronal morphology. Finally, partial nuclear colocalization between PLCβ1 and PPARγ was observed in differentiated cells, supporting the existence of a potential functional interaction between both proteins during neuronal differentiation.
Collectively, these findings support a functional association between nuclear PLCβ1 signaling and PPARγ during neuronal differentiation and suggest that PPARγ-dependent transcriptional mechanisms may participate in PLCβ1-associated neuronal fate acquisition. More broadly, these results reinforce the emerging role of nuclear lipid signaling as a regulator of transcriptional programs involved in neuronal differentiation and maturation.
Funding
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Key words: Phospholipase C beta 1; Peroxisome proliferator-activated receptor gamma; Neuronal differentiation; Nuclear lipid signaling
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Reference number: T02-12

NUCLEAR PLCB1 SIGNALING REGULATES CELL CYCLE PROGRESSION AND CDK16 REDISTRIBUTION IN NT2 HUMAN CELLS

Previous studies identified PLCβ1 as a key regulator of neuronal differentiation in NT2 cells, where its expression increases during early differentiation stages and its overexpression is sufficient to induce neuronal phenotypes even in the absence of differentiation stimuli(1). A critical event during neuronal commitment is withdrawal from the cell cycle, which is tightly associated with prolongation of the G1 phase and progressive restriction of G1/S transition. Previous work from our group demonstrated that PLCβ1 overexpression reduces proliferation and promotes G1 phase lengthening and cell cycle exit in NT2 cells, supporting a functional link between PLCβ1 signaling and cell cycle regulation during neuronal fate acquisition (2). Since cyclin-dependent kinases (CDKs) are central regulators of G1 progression and proliferation control, PLCβ1-dependent modulation of CDK-associated signaling pathways may represent a relevant mechanism coupling proliferation arrest to neuronal differentiation. Importantly, PLCβ1 contains functional nuclear localization signals, and its nuclear pool has been implicated in proliferation and differentiation control in several cellular systems. However, the contribution of nuclear PLCβ1 localization to proliferation control and CDK-associated signaling remains poorly understood.

Here, we investigated the role of nuclear PLCβ1 signaling in proliferation control and CDK-associated regulatory pathways in human NT2 cells. To this end, NT2 cells were transfected with wild-type PLCβ1 or with the nuclear localization-defective mutant PLCβ1-M2b, and proliferation was evaluated by BrdU incorporation assays. In parallel, the intracellular distribution and colocalization patterns of PLCβ1 and the cell cycle-related kinase CDK16 were analyzed by immunofluorescence microscopy and colocalization analysis.

Our results showed that PLCβ1 overexpression significantly reduced BrdU incorporation in NT2 cells, indicating decreased proliferation and promoting cell cycle exit. In contrast, expression of the nuclear localization mutant PLCβ1-M2b abolished this antiproliferative effect, indicating that PLCβ1-dependent regulation of proliferation requires correct nuclear localization. In addition, disruption of PLCβ1 nuclear localization altered the intracellular distribution of the cell cycle-related kinase CDK16, increasing its cytosolic colocalization with PLCβ1.

Collectively, these findings identify nuclear PLCβ1 signaling as a regulator of proliferation control in NT2 cells and suggest that PLCβ1 nuclear compartmentalization may influence broader CDK-associated signaling networks linked to cell cycle progression and neuronal fate acquisition.

Funding

This work was supported by grants from the University of the Basque Country (EHU-N25/14) and by the Basque Government (IT1492-22).

Key words: Phospholipase C beta 1, Cell cycle regulation, Cyclin-dependent kinase 16, Neuronal differentiation

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Reference number: T02-13

THE E3 LIGASE UBE3A REGULATES MTOR VIA DEGRADATION

Angelman Syndrome (AS) is a rare neurogenetic disease caused by the absence or dysfunction of UBE3A protein in the brain, an E3 ligase involved in protein ubiquitination. In order to understand the molecular mechanisms underlying the disease, it is essential to identify the substrates of Ube3a. However, to date, little is known about them. With the aim of identifying Ube3a substrates, we have performed a quantitative proteomics experiment to analyze the neuronal ubiquitome of mice overexpressing Ube3a [1]. We detected mTOR, a serine/threonine kinase involved in protein synthesis and synaptic plasticity [2], as a putative Ube3a substrate. Furthermore, we confirmed that human Ube3a ubiquitinates mTOR in the HEK293T cell line. Ubiquitin-related modifications control a plethora of essential cellular processes but has been linked typically to protein degradation [3]. Due to this, we performed a cycloheximide degradation assay and established that Ube3a-dependent mTOR ubiquitination results in mTOR degradation. Thus, mTOR has been validated as a degradation target of Ube3a.

Future experiments will focus on defining the downstream effects of this behavior, as well as discovering the deubiquitinases that counteract this activity.

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Reference number: T02-14

STUDYING THE IMPACT OF TAU AGGREGATION ON ASTROCYTE BIOLOGY IN ALZHEIMER'S DISEASE

Protein aggregation is a hallmark of Alzheimer's disease (AD), a neurodegenerative disorder characterized by the presence of amyloid-beta and tau aggregates in the brain. These protein aggregates deeply impact on neuronal function and integrity, finally leading to their degeneration. However, it remains unclear the alterations that astrocytes undergo when exposed to tau aggregates. Here, we aim to describe the effects that tau aggregates exert on astrocyte biology in different disease models of AD.
Mouse primary cortical astrocytes were exposed to tau-preformed-fibrils (tau-PFFs) at different timepoints (1 and 5 days) to study their impact on astrocyte biology by using immunofluorescence, live-cell calcium imaging and tau-Proximity Ligation Assay (Tau-PLA). Human brain tissue from control and AD patients of different Braak stages was used to describe the reactive astrocytes subpopulations present in the most affected brain regions along the disease, as well as to study the burden of early and late tau aggregates within them by using immunohistochemistry and Tau-PLA.
Chronic (5 days) but not acute (1 day) treatment was sufficient to impair ATP-induced endoplasmic reticulum calcium release and lysosomal pathway, as well as to increase the expression of reactivity markers in mouse primary cortical astrocytes. These effects were also extended to neurons when co-cultured with rat primary cortical neurons. Human brain tissue examination revealed region-specific differences in reactive astrocytes subpopulations and in their tau aggregates burden.
Tau pathology not only impacts on neurons but also on astrocytes, altering their biological processes and affecting to their neighbour cells. Moreover, the reactive profile of astrocytes may influence the burden of tau aggregates within them, suggesting the existence of vulnerable astrocytic subpopulations to tau pathology in AD.

Authors

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Reference number: T02-15

ENERGY PARADOX: ASTROCYTIC GLUT1 LOSS IMPROVES COGNITIVE FUNCTION IN ALZHEIMER'S DISEASE

Cerebral glucose hypometabolism is one of the earliest hallmarks of Alzheimer's disease (AD), preceding clinical symptoms by years. GLUT1, the predominant glucose transporter in astrocytes, makes these cells critical regulators of brain energy metabolism. Given this, we hypothesized that astrocyte-specific GLUT1 deletion would accelerate AD progression.

Contrary to this prediction, GLUT1 ablation significantly reduced mortality in APP/PS1 mice and fully rescued their cognitive deficits in the Morris Water Maze and Fear Conditioning tasks — without altering amyloid burden or Aβ levels. Synaptic structural integrity was equally preserved, as multi-parameter quantification of dendritic spines using a fluorescent Sindbis virus to sparsely label individual neurons revealed no significant differences across groups.

To determine whether these effects were accompanied by morphological changes, astrocytic processes were traced using Single Neurite Tracer (SNT). APP/PS1-GLUT1 KO astrocytes exhibited greater process number, total length, and branching complexity — without somatic elongation — suggesting enhanced ramification and potentially broader vascular coverage. Consistently, PET imaging revealed that astrocytic GLUT1 removal restores the global brain hypometabolism characteristic of early AD. This metabolic reprogramming extended beyond the brain: APP/PS1 GLUT1 KO mice also displayed improved glycemic control (GTT and ITT), increased carbohydrate utilization, and more efficient energy expenditure, as evidenced by thermography and brown adipose tissue PET (BAT-PET) imaging.

Beyond these metabolic findings, we investigated the cellular mechanisms underlying the cognitive rescue. GLUT1-deficient astrocytes exhibited elevated Ca^{2+} event frequency and duration. Fiber photometry corroborated a consistent trend toward increased event duration and AUC in GLUT1 KO animals — even in the presence of AD pathology — both at baseline and following an aversive stimulus, pointing to enhanced astrocytic Ca^{2+} signaling. Critically, chemogenetic suppression of this activity completely abolished the cognitive rescue, establishing astrocytic Ca^{2+} dynamics as the central mechanistic driver.

Collectively, these findings reveal that astrocytic GLUT1 deletion triggers a broad adaptive response spanning morphology, Ca^{2+} signaling, and systemic metabolism — ultimately preserving cognitive function and survival in a mouse model of AD.

Authors

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Reference number: T02-16

IMPACT OF THE LRRK2 G2019S MUTATION ON ALPHA-SYNUCLEIN OLIGOMER ACCUMULATION AND MICROGLIAL ACTIVITY IN PARKINSON'S DISEASE

Parkinson’s Disease (PD) is influenced by genetic and biological variables, including the G2019S mutation in LRRK2. Given the enhanced kinase activity associated with this mutation, its role in a-synuclein (a-syn) pathology, neuroinflammation, and the emerging evidence of gender-associated differences in PD, a more detailed mechanistic

understanding of the effects of this mutation is required. This study aims to determine how the LRRK2 G2019S mutation influences a-syn oligomer accumulation and the related microglial response across several brain regions relevant in PD, considering the effects of age and gender, addressing the underrepresentation of female LRRK2 G2019S carriers in research.
Male and female LRRK2 wildtype and G2019S knock-in mice aged 12 or 48 weeks were examined. Quantitative analysis of a-syn oligomeric load, alongside microglial morphology and autophagy lysosomal pathway assessment were conducted on the motor cortex, dorsal striatum and the substantia nigra pars compacta using immunohistochemistry and a-syn Proximity Ligation Assay (AS-PLA).
The G2019S mutation altered the number and distribution of a-syn oligomers, with gender-specific patterns emerging across brain regions, especially at 48 weeks of age. Microglial phenotypes also shifted, including changes in morphology, a-syn oligomeric content and in the autophagic machinery, as reflected by alterations in p62 and LAMP1 levels, which also exhibited clear gender- and age-dependent signatures.
The impact of the LRRK2 G2019S mutation on a-syn oligomer pathology and microglial behaviour is strongly influenced by both age and gender. These findings underscore the importance of considering gender as a key analytical variable in LRRK2 associated PD research and emphasize the importance of identifying gender-specific mechanisms that may drive disease onset and progression in LRRK2 G2019S carriers.

Authors

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Reference number: T02-17

MOLECULAR AND MORPHOLOGICAL ALTERATIONS IN OLIGODENDROCYTE LINEAGE CELLS IN HUMAN AND SNCA-OVX MOUSE MODELS IN PARKINSON'S DISEASE

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the gradual loss of dopamine-producing neurons in the substantia nigra pars compacta. Oligodendrocyte progenitor cells (OPCs) are found in the central nervous system (CNS) that can differentiate into oligodendrocytes, the cells responsible for myelinating axons in the CNS. Studies have suggested that OPCs may have a range of functions beyond myelination, including neuroprotection, and synaptic plasticity. The role of OPCs in PD is still unknown, however studies have shown that human single-cell transcriptome analysis from both SN and cortex have a large population of OPCs in PD subjects, and that PD genetic risk correlates better with oligodendrocyte lineage cells (OLs) than other cell types in SN.
Here we examined the expression of a snRNAseq dataset from control/PD human samples through Gene Set Enrichment Analysis. In PD samples, pathways related to oxidative stress and heat-shock proteins were found upregulated in oligodendrocytes. Interestingly, we found a significant increase in the expression of myelination-associated genes in a SNCA-overexpressing (SNCA-OVX) mouse model of PD. Therefore, we further explored the biology of cells of OL in human tissue and SNCA-OVX animals. We found an age and brain region-dependent increase in the number and size of OPCs, and also detected an increase in oligomeric α-synuclein aggregates in the processes of PDGFRα-

positive cells in SNCA-OVX animals, whereas in control animals, these aggregates were preferentially observed in the soma. Additionally, the cingulate cortex of PD patients showed a larger myelinated area and a more circular morphology. In SNCA-OVX animals, we also observed an increasing trend in the myelinated area of the cortex.
Our results from both human and SNCA-OVX mice suggests that α-synuclein pathology may influence oligodendrocyte-driven myelination changes, both in SN and cortex. Further research is needed to determine the functional relevance of these findings in PD pathophysiology.

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Reference number: T02-18

APOE4 HUMAN OLIGODENDROCYTES GENERATE ALTERED MYELIN AND IMPAIR AXON–MYELIN ORGANIZATION IN CHIMERIC MICE

Oligodendrocytes (OLs) are the myelinating glial cells of the central nervous system and are essential for the efficient conduction of electrical impulses. OL dysfunction and myelin degeneration are increasingly recognized as features of Alzheimer's disease (AD), although the underlying mechanisms remain poorly understood. APOE4, the strongest genetic risk factor for sporadic AD, disrupts cholesterol homeostasis and has been associated with impaired myelination. While most studies addressing these mechanisms have relied on animal models, species-specific differences highlight the need to investigate human OL (hOL) biology directly.
Here, we generated and characterized OLs derived from isogenic APOE3/3 and APOE4/4 human induced pluripotent stem cell (hiPSC) lines. At DIV75, hiPSC-derived cells were transplanted into the corpus callosum of immunodeficient Rag2^{-}/$^{-}$ Mbpshi/shi (shiverer) mice. Nineteen weeks after transplantation, human cells had proliferated and extensively migrated throughout the mouse brain, with the majority differentiating into the oligodendroglial lineage, as confirmed by OLIG2 and SOX10 immunostaining. In the corpus callosum and other fiber-rich regions, hOLs robustly myelinated the host brain, as demonstrated by extensive MBP staining. In contrast, cortical regions contained scattered MBP+ cells together with MBP−BCAS+ cells displaying intermediate stages of differentiation.
Quantification of MBP integrated density in the corpus callosum revealed reduced myelin formation by APOE4/4 hOLs compared with APOE3/3 hOLs. Moreover, transplantation of hOLs promoted the reorganization of nodal (Nav1.6) and paranodal (CASPR) proteins in the host brain, leading to the formation of well-defined nodes of Ranvier. Comparative analysis of nodal architecture between APOE3/3 and APOE4/4 chimeric mice showed reduced Nav1.6 nodal length in APOE4/4-transplanted animals. Together, these findings suggest that the APOE4 genotype impairs both myelin formation and axon–myelin domain organization in hOLs in vivo. Ongoing studies aim to further elucidate how APOE4 affects myelination and cholesterol homeostasis in OLs using chimeric mouse models.
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Reference number: T02-19

OPPOSING EFFECTS OF APOE3 AND APOE4 HUMAN ASTROCYTES ON ALZHEIMER'S DISEASE HALLMARKS IN CHIMERIC MICE

Astrocytes and apolipoprotein E (APOE) are strongly implicated in the pathogenesis and progression of Alzheimer's disease (AD). However, the impact of astrocytes carrying different APOE variants on core AD hallmarks remains poorly understood. To bridge this gap, we generated a chimeric model of AD by transplanting isogenic APOE3 or APOE4 human induced pluripotent stem cell (hiPSC)-derived astrocyte progenitors, into the neonatal brains of AD mice. Five to six months post-transplantation, engrafted cells predominantly integrated into the upper layers of one cortical hemisphere and differentiated into mature astrocytes. Notably, these cells adopted the morphology of interlaminar astrocytes (h-iAstrocytes), a human-specific astrocyte population whose role in AD remains unexplored. Interestingly, APOE3 and APOE4 h-iAstrocytes drove opposing effects on core AD hallmarks. APOE3 h-iAstrocytes ameliorated Aβ and Tau pathologies as well as neuritic dystrophy. In contrast, APOE4 h-iAstrocytes exacerbated these processes. The two isoforms also induced opposing microglial responses to Aβ pathology: APOE4 h-iAstrocytes increased microglia clustering around Aβ plaques, and promoted a disease-associated microglia (DAM)-like state, whereas APOE3 h-iAstrocytes reduced clustering and supported a more homeostatic profile. These findings reveal that h-iAstrocytes critically influence multiple AD pathological hallmarks in chimeric mice and demonstrate that APOE variants modulate astrocyte biology playing a pivotal role in AD progression. We are now generating additional chimeras with astrocyte progenitors carrying other AD risk variants to define how diverse genetic backgrounds contribute to disease pathology. We are also studying the spatial interactions between human astrocytes and host cells to define the mechanisms underlying pathological outcomes.

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Reference number: T02-20

KETOGENIC DIET AS A THERAPEUTIC STRATEGY TO COUNTERACT THE IMPACT OF ASTROCYTIC INSULIN RECEPTOR DELETION IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is characterized by progressive cognitive decline and profound alterations in brain metabolism and neuronal network activity.While research has traditionally focused on neuronal dysfunction, increasing evidence highlights the critical role of astrocytes in regulating brain energy homeostasis and neuronal excitability. In this context, astrocytic insulin receptor (IR) signaling has recently emerged as a key contributor to AD pathogenesis. Indeed, previous results from our laboratory proved that astrocyte-specific IR deletion in mice causes decreased brain glucose utilization, aberrant astrocytic calcium dynamics, reduced ATP release, and neuronal

hyperexcitability which leads to memory impairment and accelerates the progression of AD pathology.
Building on these findings, we hypothesize that the reduction of astrocytic ATP release is reflected in a reduced extracellular adenosine. Since adenosine-mediated inhibition is a key mechanism for dampening neuronal excitability, this diminished inhibitory tone may contribute to the neuronal hyperexcitability observed in these mice. We further propose that a ketogenic diet could drive an astrocytic metabolic shift capable of restoring adenosine release, strengthening adenosine-mediated inhibition, and ultimately reducing the aberrant neuronal hyperexcitability observed in our mouse model.
To test this, we employed four genetically distinct mouse groups: controls, astrocyte-specific IR knockout mice (IRΔGFAP), AD mice (APP/PS1), and AD astrocyte-specific IR knockout (APP/PS1-IRΔGFAP). Beginning at 2 months of age, animals from each genotype were assigned to either a standard diet or a ketogenic diet for a period of two months. At 4 months of age, when mice maintained on a standard diet already exhibit cognitive impairment, we assessed cognitive function together with peripheral metabolic status. Our results show that the ketogenic diet was able to reverse the cognitive deficits observed in IRΔGFAP mice; however, it failed to rescue the severe impairment detected in APP/PS1-IRΔGFAP animals.
Overall, these findings suggest that ketogenic diet-induced metabolic reprogramming may partially compensate for the consequences of astrocytic IR deficiency, improving cognitive performance and metabolic alterations. These results further support a role for astrocyte-dependent purinergic signaling in the regulation of neuronal excitability and cognitive decline during AD progression.

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Reference number: T02-21

MYELIN-DERIVED FATTY ACIDS AMELIORATE PATHOLOGY IN HUMAN CORTICAL ORGANOIDS FROM ALZHEIMER'S DISEASE

Age-related metabolic decline profoundly affects brain function, and increasing evidence indicates that myelin structure and metabolism are altered during aging. In Alzheimer's disease (AD), where aging is the strongest risk factor, impaired myelin metabolic support may contribute to neurodegeneration. Building on our recent findings that myelin can function as an energy reservoir (Nat Metab, 2025; 10.1038/s42255-025-01244-7), we investigated whether deficits in myelin-derived metabolic substrates exacerbate AD pathology. Human cortical organoids were generated from induced pluripotent stem cells (iPSCs) derived from a female patient with sporadic AD. Organoids were validated for regional identity and maturation using neuronal and glial markers (SOX2, MAP2, TUJ1, CTIP2, S100β). Key pathological features—including oxidative stress, tau hyperphosphorylation, astrocyte reactivity, inflammatory signaling, and synaptic integrity—were assessed by immunofluorescence and RT-qPCR. Exposure of 4-month-old AD organoids to myelin-derived fatty acids significantly reduced redox-sensitive imaging signals, Nrf2-associated inflammatory responses, phospho-tau levels, and astrogliosis, while increasing the expression of functional astrocytic markers and synaptic proteins. Ongoing studies using calcium imaging and microelectrode arrays (MEAs) are evaluating the impact of these treatments on neuronal network activity. In

parallel, 2D human astrocyte cultures are being used to investigate the metabolic regulation of oxidative stress and reactive phenotypes. Together, these findings suggest that myelin-derived fatty acids can mitigate AD-related pathology in human brain organoids and identify aged myelin metabolism as a critical modulator of disease progression.

Authors

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Reference number: T02-22

METABOLIC FLEXIBILITY OF CNS CELLS UNDER GLUCOSE DEPRIVATION

The brain, despite accounting for only 2% of body weight, consumes a disproportionate amount of energy and relies largely on glucose to sustain its high metabolic demand. Preserving energy homeostasis is essential for proper central nervous system (CNS) function, and reduced metabolic flexibility has been linked to neurodegenerative processes. Long-chain fatty acids represent a potential alternative substrate for mitochondrial metabolism, as their oxidation yields high amounts of ATP and requires mitochondrial import via carnitine palmitoyltransferase 1A (CPT1A). Here, we examined how fatty acid availability influences metabolic and functional responses of CNS cells under conditions of reduced glucose availability. Using primary neurons, astrocytes, and oligodendrocytes exposed to hypoglycemia, we assessed mitochondrial function and cellular energy status in the presence of long-chain fatty acids. CPT1A expression was detected across CNS cell types, supporting their capacity to engage fatty acid–dependent metabolic pathways. Hypoglycemia induced cell-type–specific metabolic responses, with glial cells showing a relative preservation of mitochondrial activity compared to neurons, while fatty acid supplementation modulated mitochondrial parameters and mitochondrial membrane potential, suggesting altered mitochondrial coupling during metabolic stress. Because axonal conduction is highly energy dependent, we further investigated whether fatty acid metabolism can sustain axonal function under glucose deprivation. Electrophysiological recordings from acutely isolated optic nerves revealed that myelin-derived fatty acids significantly improved the recovery of evoked compound action potentials following glucose deprivation. Together, these findings demonstrate heterogeneous metabolic adaptations among CNS cell types and identify fatty acid metabolism as a key contributor to both cellular energy homeostasis and axonal function under hypoglycemic conditions.

Authors

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Reference number: T02-23

ASTROCYTES OF THE MOUSE SUPRACHIASMATIC NUCLEUS RESPOND TO GLUCOCORTICOID SIGNALING

The circadian system synchronizes physiology to 24-hr rhythms that anticipate daily environmental changes (light/dark cycles). In mammals, the hypothalamic suprachiasmatic nucleus (SCN), acting as a master pacemaker, is responsible for scheduling “wake” functions by coordinating the release of glucocorticoids (GCs) in the beginning of the active phase. Although GCs entrain peripheral clocks via GC receptor (GR) activation, the SCN has been traditionally considered resistant to GCs due to low expression of GR in adult SCN neurons. We have found that while GR is broadly

expressed in the SCN early in development, the expression is restricted mainly to astrocytes as the circuit matures. We hypothesize that SCN astrocytes respond to GC signalling depending the time of day.
To test our hypothesis, we used 2-Photon Ca2+ imaging in acute SCN slices loaded with the permeable sensor Fluo-4-AM. Astrocytic Ca2+ dynamics were recorded in basal conditions and under pharmacological treatments with corticosterone (CORT, main GC in rodents), an inhibitor of astrocytic communication (GAP 26) and a combination of both. To prevent genomic responses, the slices were incubated in artificial cerebrospinal fluid (aCSF) containing actinomycin D and cycloheximide (transcription and translation inhibitors respectively). Additionally, to assess whether CORT might be acting through the GR on the cell membrane, slices were treated with CORT-BSA to prevent the hormone to enter the cell.
CORT treatment increased the frequency of astrocytic calcium events when the slice was prepared 0-2 hrs after lights on (zeitgeber time, ZT0-2), an effect that appears to be dependent on astrocytic communication. Interestingly, this effect is stronger when the slice was prepared 0-2 hrs after lights off (ZT 12-14), and the astrocytic network appears to be more vulnerable to the inhibition of glial communication. The effect of CORT is independent of transcription and translation and seem to depend on the activation of membrane bound GR because the effects were abolished under treatment with a GR antagonist.
Overall, these findings show that astrocytes in the SCN can respond to GC signalling in a time-dependent manner. The effects are stronger at the time of day when CORT is at the peak, revealing a possible gated hormonal feedback mechanism onto the SCN.

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Reference number: T02-24

ACUTE EFFECTS OF PSILOCYBIN ON MICROGLIAL MORPHOLOGY IN BRAIN REGIONS ASSOCIATED WITH DEPRESSION

Psilocybin has demonstrated promising rapid antidepressant effects in clinical studies of major depressive disorder and treatment-resistant depression. Although the mechanisms underlying these effects remain incompletely understood, growing evidence suggests that psychedelics may influence neuroplasticity and neuroimmune-related processes. Microglia, the resident immune cells of the central nervous system, play key roles in synaptic remodeling, inflammatory signaling and regulation of the neural microenvironment, making them a potential target of psilocybin-induced neurobiological changes. In this study, we investigated the acute effects of psilocybin on microglial morphology across selected brain regions. GFP-expressing mice received psilocybin (5 mg/kg, i.p.) or saline and were perfused 3 hours after administration. Confocal microscopy was used to examine microglia within the medial prefrontal cortex (mPFC) and dentate gyrus (DG), followed by Sholl analysis to assess morphological complexity. Preliminary findings indicate region-specific alterations in microglial morphology following psilocybin administration, including increased process complexity, suggesting changes in microglial functional state. These findings support further investigation into the relationship between psilocybin, microglial dynamics, and neuroimmune-related mechanisms relevant to depression.

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Reference number: T02-25

STARS OF THE MASTER CIRCADIAN CLOCK: A CHARACTERISATION OF GLIOGENESIS IN THE MOUSE SUPRACHIASMATIC NUCLEUS

In mammals, circadian rhythms are organised by the master pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus. Astrocytes are active regulators of circadian rhythmicity, however, the developmental timing of their functional integration into the circuit remains poorly understood. Previous work has shown that in the mouse, molecular rhythms gain robustness and synchrony at a circuit level during late gestation, coinciding with the neurogenic-to-gliogenic transition. Thus, we hypothesize that astrogenesis may be critical for the functional maturation of the SCN. In this study, we characterized the temporal dynamics of SCN gliogenesis using birth-dating approaches, with a particular focus on astrocyte proliferation. Using EdU labelling in combination with cell-type specific markers we characterised numbers and distribution of newly born cells in the late gestation and early postnatal period within the mouse SCN. During that same period, we identified astrocyte-specific and astrocyte-enriched genes and characterised the expression patterns along the developmental trajectory. We found that genes related to astrocyte specification (Nfib, Nfix, Tgfb1), are sustained postnatally indicating continued proliferation. We also found that astrocytes show early maturation and integration in the SCN circuit, as shown by upregulation from the first postnatal week of genes necessary for GABA, glutamate and purinergic homeostasis (Slc12a2, Slc12a3, Slc6a11, Entp2d). Building on these insights, we used organotypic cultures to assess the robustness and synchrony of molecular rhythms when astrocyte proliferation or astrocyte functionality are selectively disrupted. Together, these results provide new insight into the importance of glial maturation for the functional assembly of the central biological clock.

Authors

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Reference number: T02-26

ALTERED PERINEURONAL NETS-OLIGODENDROCYTE DYNAMICS IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDER

Inhibitory circuit dysfunction is a hallmark of Autism Spectrum Disorder (ASD), yet how glial cells and the extracellular matrix (ECM) contribute to the maturation and maintenance of these circuits remains largely unexplored. Perineuronal nets (PNNs), specialized ECM assemblies ensheathing parvalbumin-expressing (PV+) interneurons, are critical regulators of inhibitory circuit plasticity, and we have demonstrated that disrupted myelination is linked to PV+ neuron hypofunction in an ASD model. However, whether oligodendrocyte lineage cells also participate in PNN formation remains unknown. Here, we investigated the relationship between oligodendrocytes, PNNs, and PV+ interneurons in the somatosensory cortex of cntnap2−/− mice. Transcriptomic profiling of MACS-isolated O4+ oligodendrocytes revealed a robust dysregulation of GABAergic and interferon signaling pathways, characterized by a coordinated shift in extracellular matrix (ECM) dynamics. This signature included the upregulation of PNN assembly components (Tnr, Bcan), a downregulation of PNN-degrading enzymes (Mmp15, Mmp14), and reduced levels of adhesion and signaling receptors (Itga8, Itgb4,

Cd44). At the structural level, cntnap2−/− mice displayed reduced PV+ interneuron density and fewer PNNs. High-resolution imaging revealed that the apposition of OPCs and mature oligodendrocytes to PNN+/PV+ neurons is both cell-type-specific and genotype-dependent, with with OPCs found in close juxtaposition to the PNN+/PV+ soma, whereas differentiating oligodendrocytes undergo a spatial relocation towards the dendritic and axonal compartments. Importantly, interferon-γ stimulation of oligodendrocytes in vitro upregulated tenascin-R expression. Together, these findings suggest that oligodendrocyte lineage cells are active participants in PNN biology and may be implicated in the inhibitory circuit alterations observed in this ASD model.

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Reference number: T02-27

PTZ-INDUCED NEURONAL HYPEREXCITABILITY DISRUPTS GLIAL HOMEOSTASIS IN ZEBRAFISH LARVAE THROUGH TRKB-BDNF PATHWAY

Neuronal hyperexcitability is an early hallmark of Alzheimer's disease (AD), yet its impact on glial dynamics and oligodendroglial maturation remains poorly defined. We modeled hyperexcitability in 5 dpf zebrafish larvae using chronic exposure to 2 mM pentylenetetrazole (PTZ), a GABA A receptor antagonist that increases neuronal excitability.

PTZ induced no toxicity but produced robust increases in calcium spike amplitude and frequency in the optic tectum, confirming sustained hyperexcitability. Behaviorally, PTZ-treated larvae exhibited strong locomotor hyperactivity and enhanced anxiety-like behavior. At 5 dpf, double labelling in Tg(sox10:RFPt) and Tg(olig2:EGFP) larvae revealed no changes in total oligodendrocyte number, but showed a clear increase in $sox10^{+}/olig2^{+}$ mature oligodendroglial cells. This shift toward a more mature profile was corroborated by RT-qPCR upregulation of different oligodendrocyte maturation markers. Microglial number remained unchanged, but PTZ induced clear microglial activation, including amoeboid morphology and increased phagocytic activity. PTZ also reduced microglia–oligodendrocyte interactions, suggesting impaired glial communication. Consistently, similar reductions were observed in $p2y12^{+/-}$ larvae, suggesting that this impairment is partially mediated by P2Y12-dependent microglial activity sensing. Interestingly, at 8 dpf larvae previously exposed to PTZ displayed a reduction in total $olig2^{+}$ oligodendrocytes, indicating that early hyperexcitability ultimately disrupts oligodendrocyte lineage progression. Finally, treatment with the TrkB inhibitor ANA 12 fully reversed PTZ-induced locomotor hyperactivity, anxiety-like behavior, and oligodendroglial maturation changes, demonstrating that TrkB–BDNF signaling is a key pathway linking neuronal hyperexcitability to glial dysfunction.

Together, these findings show that early neuronal hyperexcitability disrupts glial homeostasis by activating microglia, impairing microglia–oligodendrocyte interactions, and altering oligodendrocyte maturation, ultimately compromising lineage progression. The full rescue of behavioral and cellular phenotypes by ANA 12 identifies TrkB–BDNF signaling as a key pathway linking hyperactive neural states to glial dysfunction, providing mechanistic insight relevant to early stages of AD.

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Reference number: T02-28

POLYPHENOL-BASED MODULATION OF AMYLOID PATHOLOGY AND NEUROINFLAMMATION IN 5XFAD ALZHEIMER'S DISEASE MOUSE MODEL

Alzheimer's disease (AD) is the leading cause of dementia and is characterized by amyloid-beta (Aβ) plaques and tau hyperphosphorylation. Although the amyloid cascade hypothesis has dominated the field for decades, early events such as synaptic dysfunction and glial alterations are increasingly recognized as critical contributors to cognitive decline. In this context, natural polyphenols have emerged as promising therapeutic agents due to their potential to modulate both amyloid pathology and neuroinflammation.

In this study the effects of a polyphenol-rich plant extract in the 6-month-old 5XFAD transgenic mouse model, which presents early and aggressive Aβ deposition were evaluated. Brain cortex samples from male and female 5XFAD and wild-type (WT) mice were studied following oral administration of the polyphenolic extract or vehicle. Cortical Aβ42 levels were quantified by ELISA, while amyloid plaque accumulation was assessed using thioflavin-S staining and immunohistochemistry. In both analyses, a reduction in the amyloid pathology in male 5XFAD mice treated with the extract compared to the 5XFAD mice treated with vehicle was observed. However, in 5XFAD female mice, changes were only detected in cortical Aβ42 levels. Glial responses were evaluated by immunofluorescence against Iba1 and GFAP. Interestingly, the treatment presented a sex-dependent effect. In males, the extract showed a trend toward reduced microgliosis, astrogliosis, and neuroinflammation, whereas females displayed more moderate or even opposite trends, reinforcing the presence of sexual dimorphism in therapeutic response. In addition, synaptic alterations were examined by western blot using PSD95 and synaptophysin as markers. Both synaptic proteins exhibited partial recovery toward WT levels.

Overall, these findings support the potential of plant-derived polyphenols as modulators of AD-related pathology and neuroinflammatory processes. However, the observed sexual dimorphism suggests that the therapeutic window may differ between sexes. Since female 5XFAD mice exhibit a more aggressive phenotype at 6 months, treatment may need to be administered at an earlier stage of the disease to achieve neuroprotective efficacy.

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Reference number: T02-29

MYELIN BASIC PROTEIN OVEREXPRESSION IMPAIRS BEHAVIOR IN THE WILD TYPE MOUSE AND TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER´S DISEASE DURING EARLY STAGES OF PATHOLOGY

Oligodendrocytes (OLs) are responsible for generating myelin sheaths around axons, which enables fast and efficient nerve conduction and is crucial for the proper cognitive functioning of the central nervous system. In Alzheimer's disease (AD), changes in myelinated axons have been observed, indicating that myelin dysfunction could be an important aspect of the disease's pathophysiology. Myelin Basic Protein (MBP), the primary constituent of myelin, is dysregulated in AD models. Based on this, we hypothesize that the overexpression of MBP might lead to OL dysregulation and myelin abnormalities in AD patients and in wild type and AD mouse models.

First, MBP expression analysis in the hippocampus of healthy and AD individuals revealed a significant increase in MBP levels in late-stage AD patients (CERAD C) compared to controls. Immunofluorescence assays showed that this upregulation was specifically localized to the dentate gyrus and CA3 regions, whereas no changes were observed in CA1 or fimbria.

Next, overexpression of MBP in cultured OLs using the adeno-associated viral (AAV)-vectors driven by the MBP promoter; AAV8-MBP promoter-MBP-IRES-GFP (AAV-MBP-GFP) showed increased MBP mRNA and protein levels compared to control cells infected with AAV8-MBP promoter-GFP (AAV-GFP). MBP overexpression did not affect oligodendroglial viability but altered calcium influx in response to plasma membrane depolarization and mitochondrial energetic metabolism.

To assess the in vivo effects, we stereotactically injected AAV vectors into the dentate gyrus of the hippocampus in the WT and 3xTg-AD mice at 5 and 12 months of age and, after 3 months, behavioral assessments were conducted. At 8 months of age, WT and 3xTg-AD mice injected with AAV8-MBP-GFP showed increased latency in Barnes maze. Moreover, anxiety-related behaviors were altered only in 3xTg-AD mice injected with AAV8-MBP-GFP. However, by 15 months of age, no changes were observed across any of the groups. Ultimately, 3xTg-AD mice injected with AAV8-MBP-GFP exhibited a lower g-ratio than mice injected with the control virus by electron microscopy.

In conclusion, we have established a novel tool to further study the role of MBP overexpression both in vivo and in vitro. Importantly, our preliminary results suggest that overexpression of MBP during the early stages of the disease alters the behavior of 3xTg-AD mice, highlighting its early detrimental effects. However, further research is needed to elucidate the mechanisms underlying myelin-associated alterations in AD.

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Reference number: T02-30

MYELIN PLASTIC CHANGES FOLLOWING EXPOSURE TO HIGH-SEVERITY STRESS

Myelin sheaths support the fast, saltatory conduction of action potentials along axons. Beyond the role in development, experience and neuronal activity have been shown to drive dynamic changes in myelination, a process now recognized as central to learning,

memory consolidation and social behavior. Previous work from our group demonstrated that chronic chemogenetic activation of mature oligodendrocytes alters innate emotional behavior and impairs the extinction of aversive memories in female mice, suggesting that maladaptive myelination may shape emotional and stress-related responses. To test the reverse hypothesis, we asked whether post-traumatic stress could itself remodel myelin. We used immobilization on boards (IMO) in mice as a model of post-traumatic stress disorder (PTSD), a paradigm that reliably induces behavioral and biological changes recapitulating core PTSD features. Using immunofluorescence and confocal microscopy, we analyzed myelin organization in brain regions involved in sensory processing and emotional regulation. Stressed mice showed a significant reduction in myelinated fiber density in the amygdala, accompanied by a shortening of the nodes of Ranvier as revealed by STED microscopy, with no changes in the proportion of mature oligodendrocytes or progenitors of oligodendrocytes, pointing to a selective impact on myelin structural organization rather than on oligodendrocyte lineage dynamics. To test whether enhancing myelination could rescue the behavioral phenotype, we administered clemastine, a pro-myelinating compound, to PTSD mice. Clemastine treatment did not restore fear extinction, suggesting that promoting de novo myelination is insufficient to reverse the behavioral impairment, and pointing to nodal remodeling, rather than myelin sheath formation per se, as a potentially critical substrate of PTSD-related circuit dysfunction.

Authors

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Reference number: T02-31

EARLY DISRUPTION OF OLIGODENDROCYTE HOMEOSTASIS AND NEURONAL HYPEREXCITABILITY IN A SEX- AND REGION-DEPENDENT MANNER IN 3XTG-AD MICE

Alzheimer's disease (AD) is characterized by progressive cognitive decline preceded by early functional alterations in neuronal circuits. Increasing evidence suggests that neuronal hyperexcitability emerges at early stages of the disease and may contribute to network instability and disease progression.

In previous work using the 3xTg-AD mouse model, we reported a reduction in oligodendrocyte populations at 12 months of age. However, it remains unclear whether these alterations are already present during earlier stages of pathology and whether they are associated with changes in neuronal activity.

Here, we investigated early alterations in oligodendroglial populations and neuronal activity in 3xTg-AD mice, and explored their potential relationship during disease progression. We focused on limbic system-related regions, including the hippocampus, amygdala and prefrontal cortex, given their early vulnerability in AD. Analyses were performed at 3 and 6 months of age in 3xTg-AD mice using immunofluorescence, stereological and behavioral approaches to assess oligodendrocyte lineage cells and neuronal activity.

Our results show that oligodendroglial alterations are already present at early stages, with a significant reduction in oligodendrocyte populations observed at both 3 and 6 months of age. At 3 months, we identified a sex- and region-dependent vulnerability. In

females, oligodendrocyte precursor cells were reduced in the hippocampus and amygdala, whereas an increase was observed in the prefrontal cortex. No significant changes were detected in males, indicating a sex-specific pattern in early oligodendroglial dynamics.
Importantly, these cellular alterations were accompanied by increased neuronal activity in the same regions, consistent with early network hyperexcitability. These findings indicate a coordinated alteration of neuronal and glial homeostasis during disease onset, suggesting that early circuit dysfunction is associated with oligodendrocyte dysregulation.
Overall, our results indicate that oligodendroglial alterations arise early in 3xTg-AD mice and could be associated with increased neuronal activity, highlighting a functional interaction between both processes during the initial stages of AD.

Authors
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Reference number: T02-32

HYPOXIA AND ELEVATED PRESSURE DIFFERENTIALLY ALTER MITOCHONDRIAL DYNAMICS AND METABOLISM IN ADULT MÜLLER GLIA

The retina is highly vulnerable to hypoxia and elevated intraocular pressure, two major stressors involved in glaucoma. Müller glia (MG), the principal retinal glial cells, are essential regulators of retinal metabolism and neuronal homeostasis. Mitochondrial transfer from MG to RGCs via extracellular vesicles (EVs) has recently emerged as a potential neuroprotective mechanism. Here, we investigated how hypoxia and high pressure (HP) affect MG metabolism, focusing on mitochondrial function and EV-mediated communication.
Primary adult rat MG were cultured on poly-L-lysine- and laminin-coated plates under hypoxia (1% O_2) or elevated pressure (+70 mmHg) for 72 h. Mitochondrial content was quantified using VDAC1 immunolabeling. Mitochondrial-related gene expression was analysed by RT-qPCR in both MG and derived EVs, including genes involved in oxidative stress (SOD2), mitochondrial biogenesis (NRF1), mitochondrial fission (DRP1), mitophagy (PINK1), and mitochondrial electron transport chain function (COX1, CYTB, ND1).
Hypoxia increased mitochondrial density in MG (0.0488 to 0.0907 mit/μm^2), whereas elevated pressure reduced mitochondrial number (0.1108 to 0.0854 mit/μm^2), indicating distinct mitochondrial responses to retinal stress. Under hypoxia, MG exhibited decreased SOD2 and COX1 expression together with increased PINK1, CYTB, and ND1 levels, suggesting an adaptive mitochondrial remodelling response associated with enhanced mitophagy and maintenance of mitochondrial activity under metabolic stress. In contrast, elevated pressure induced downregulation of PINK1 and mitochondrial electron transport chain-related genes (CYTB, COX1, ND1), consistent with mitochondrial dysfunction and reduced metabolic capacity.
Analysis of EV-associated transcripts further revealed stress-dependent differences in extracellular metabolic signalling. EVs derived from hypoxic MG displayed selective enrichment of mitochondrial-associated transcripts despite reduced SOD2 levels,

whereas EVs from pressure-exposed MG showed broad upregulation of all analysed genes, suggesting distinct mechanisms of glial adaptation and intercellular communication under different pathological conditions.
These results suggest that MG respond to hypoxia through a compensatory mitochondrial remodeling program, while elevated pressure induces mitochondrial dysfunction and metabolic decline. The differential regulation of mitochondrial-associated transcripts in EVs further supports the idea that MG adapt their extracellular metabolic signaling according to the type of retinal stress.
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Reference number: T02-33

SHEAR STRESS DRIVES MÜLLER GLIA SURVIVAL AND EARLY FIBROTIC REMODELLING VIA TRPV4 AND TGF-B1

In retinal pathologies such as retinal detachment and epiretinal membranes, shear stress at the subretinal and/or vitreoretinal interface contributes to fibrotic remodelling. Müller glia (MG), are mechanosensitive cells exclusive to the retina. This study investigated the effects of flow-induced shear stress on MG survival, morphology, and extracellular matrix (ECM) remodelling, as well as the involvement of mechanosensitive pathways.
Primary adult rat MG were cultured in single-channel microfluidic slides coated with poly-L-lysine and laminin, and exposed to a 10^{-3} dyn/cm^2 shear stress for 24h. Immunocytochemistry was used to evaluate morphology (vimentin), ECM deposition (collagen I, collagen IV, fibronectin), and expression of mechanotransduction-related proteins (TRPV4, pFAK, TGF-β1). Pharmacological inhibitors of TRPV4 (HC-067472) and TGF-β1 (SB-431542) were applied to assess their functional roles. Cells were manually quantified. Cell area was measured using the freehand selection tool in ImageJ, while protein expression and ECM deposition were quantified by integrated density analysis.
Shear stress significantly increased MG survival (130.12±1.07%) compared to static conditions (100%) while reducing cell area (61.29±1.63%). TRPV4 expression was upregulated under flow (134.31±1.16%), and its inhibition reduced survival and reversed morphological changes. pFAK levels increased under flow (159.49±5.39%) in a TRPV4-dependent manner, and organised as puncta. TGF-β1 expression was elevated (166.21±3.09%), and its inhibition decreased MG survival under both static and flow conditions. ECM remodelling was selectively regulated: intracellular collagen I and IV levels increased under flow (144.93±4.26% and 193.05±4.38%, respectively) without extracellular deposition, while fibronectin deposition was significantly enhanced (189.52±6.60%) in a TGF-β1-dependent manner.
These findings demonstrate that MG are highly mechanosensitive cells that integrate shear stress signals to regulate survival, cytoskeletal remodelling, and selective ECM production. TRPV4 and TGF-β1 act as key mediators of these responses, suggesting that early mechanotransduction events may drive retinal fibrotic processes. Targeting these

pathways could provide new therapeutic strategies to prevent glial scarring and preserve retinal integrity.

Authors

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Reference number: T02-34

PERIPHERAL MÜLLER GLIA EXHIBIT ENHANCED PLASTICITY UNDER NEURAL DIFFERENTIATION CONDITIONS

Müller glia (MG) are the principal glial cells of the retina and exhibit remarkable cellular plasticity in response to injury. Previous studies have shown that peripheral MG display a greater propensity for dedifferentiation than central MG, including higher expression levels of stem/progenitor-associated markers. In this study, we investigated the differentiation potential of porcine MG isolated from central and peripheral retinal regions under neural induction conditions.

Primary porcine MG cultures were subjected to differentiation protocols adapted from stem cell differentiation models to promote neural-like phenotypic changes and sphere formation. After 21 days under differentiation conditions, cultures were analysed by immunocytochemistry to assess the expression of MG markers (vimentin and GFAP), stem/progenitor-associated markers (α-SMA, nestin and β-catenin), and neuronal-associated markers (β-III-tubulin, NeuN, ankyrin-G and VGLUT). Marker expression was quantified in monolayer-forming cells by integrated density analysis using Fiji/ImageJ software. In parallel, morphological changes associated with cellular reprogramming were evaluated.

Preliminary results revealed pronounced morphological remodelling in differentiated cultures, including the formation of neurosphere-like structures, which were predominantly observed in peripheral MG cultures. Consistently, differentiated MG showed altered expression patterns of glial and stem/progenitor-associated markers, suggestive of partial acquisition of a dedifferentiated phenotype. Notably, peripheral MG exhibited a higher degree of plasticity than central MG under identical conditions, reflected by a more robust upregulation of stem/progenitor- and neuronal-associated markers following differentiation.

Overall, these findings support the existence of region-dependent differences in MG plasticity and suggest that peripheral MG may possess enhanced regenerative potential. Ongoing studies focused on a more detailed characterization of neuronal-associated marker expression and functional differentiation will be required to better define the neurogenic capacity of these cells.

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Reference number: T02-35

ADOLESCENT BINGE DRINKING INDUCES PERSISTENT REMODELLING OF THE DENTATE GYRUS NEUROGENIC NICHE: PARTIAL STRUCTURAL RECOVERY BY OMEGA-3 FATTY ACIDS

Our previous studies demonstrated that adolescent binge-like ethanol exposure impairs CB1 receptor-dependent synaptic plasticity in the medial perforant path[1], which conveys excitatory input from the medial entorhinal cortex to the dentate gyrus (DG), a hippocampal region characterized by persistent adult neurogenesis within the subgranular zone (SGZ). Here, we investigated structural alterations in the DG induced by adolescent binge-like ethanol exposure, assessing their association with

neuroinflammatory remodelling and the potential mitigating effects of an anti-inflammatory omega-3 (n-3)-enriched diet.
Adolescent male C57BL/6J mice were exposed to a 4-week Drinking-in-the-Dark paradigm and subsequently maintained on an n-3-enriched dietary regimen during a 2-week withdrawal period. Ethanol-exposed animals exhibited a marked reorganization of the DG glial microenvironment, characterized by microglial process retraction and hypertrophic-like astroglial remodelling, consistent with a sustained neuroinflammatory-like state. In parallel, the expression of the neurotrophic marker BDNF, neuronal arborization in immature doublecortin-positive granule neurons, and hippocampal volume were significantly reduced. Notably, the n-3-enriched diet restored microglial morphology and increased the density of BDNF-expressing immature neurons, indicating partial structural recovery of the neurogenic niche, while also restoring hippocampal volume. By contrast, astroglial alterations persisted after prior ethanol exposure, indicating cell type-selective effects of the n-3-enriched diet. Extending previous evidence that n-3 supplementation enhances novel object recognition test (NORT) performance in alcohol-naïve mice[2], the present data show that the n-3-enriched diet increased discrimination performance in the NORT, including in ethanol-exposed animals. Overall, these findings suggest that adolescent binge-like ethanol exposure induces persistent neuroinflammatory remodelling of the DG neurogenic niche, associated with coordinated structural alterations in glial cells and neurons, whereas n-3 fatty acids partially counteract these alcohol-induced effects.
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Reference number: T02-36

A NOVEL ROLE FOR PROTEOLIPID PROTEIN (PLP) IN EXTRACELLULAR VESICLE UPTAKE AND COMMUNICATION IN THE CNS

Extracellular vesicles (EVs) are nanovesicles that participate in intercellular communication, playing key roles in both physiological and pathological conditions in the central nervous system (CNS). Recent studies have suggested the regenerative potential of stem cell-derived EVs in CNS pathologies. However, the biological mechanisms regulating their uptake and biological effects remain unclear. Previous studies from our laboratory suggest that EVs secreted by human neural rosettes (hNR-EVs) enriched in neural stem cells promote neurite outgrowth and maturation in human and murine neurons. Intriguingly, these trophic effects might be mediated by an unexpected neuroglial cargo associated with hNR-EV: the major proteolipid protein (PLP). While PLP is the main structural protein of CNS myelin, it is also expressed in neurons, where its functional role remains poorly understood. Beyond its structural relevance, PLP is involved in EVs biogenesis and, as a multi-pass transmembrane protein, can establish both cis- and trans-oligomeric complexes. Based on these

observations, we hypothesize that PLP facilitates EVs internalization via trans-interactions that drive its uptake by recipient neurons. In this work, we applied Stimulated Emission Depletion (STED) nanoscopy to characterize PLP distribution at the single-EV level and its association across distinct EVs subpopulations. To assess its role in EVs uptake we performed functional inhibition assays in hNR-EVs and recipient neurons using antibodies targeting an N-terminal domain of PLP. Blocking PLP in EVs and neurons significantly reduces EV uptake and neurite outgrowth. These results indicate that PLP contributes to neuronal internalization of EVs and their biological effects. Overall, our findings indicate that PLP might play an active role in EV-mediated communication in the CNS and reveal a previously unrecognized function of PLP beyond its classical role in myelination.

Authors

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Reference number: T02-37

EXPLORING THE MOLECULAR BASES OF CTNNB1 NEURODEVELOPMENTAL SYNDROME

CTNNB1 syndrome is a rare neurodevelopmental disorder, which manifests in children with symptoms such as intellectual disability, motor and speech delay, microcephaly, visual defects and autism-like behaviour. It is caused by germline de novo mutations in the CTNNB1 gene, which codes for beta-catenin, an elongated protein consisting of helical repeats. Beta-catenin is critical in development of the nervous system, based on two main functions: a transcription regulatory role in the context of the Wnt/beta-catenin signalling pathway, and a mechanical role, being part of adherens junctions (AJ) complexes. AJ-mediated adhesion provides mechanical support to neuroprogenitor tissues and is critical for brain development, as well as synapses formation and plasticity. The most prevalent CTNNB1-associated mutations generate truncated variants of beta-catenin that probably result in loss of protein function. However, the precise consequences of the pathogenic mutations on the structure and functionality of beta-catenin are presently unknown. In order to explore the effects of CTNNB1 mutations on beta-catenin folding, stability and interaction with certain ligands, we are expressing recombinant variants corresponding to various patients, and characterizing their conformational properties through circular dichroism spectroscopy and size exclusion chromatography. Our data indicate that the truncated variants can adopt to some extent a native-like secondary structure but their thermal stability is significantly altered. Moreover, to explore the putative misregulation of cell adhesion during brain development in CTNNB1 syndrome, we are evaluating the consequences of CTNNB1-linked mutations on beta-catenin interaction with N-cadherin. Our results suggest that cadherin binding is impaired for some variants.

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Reference number: T02-38

TIER-SPECIFIC LOCATION OF LEWY BODY PATHOLOGY AND RELATED NEUROMELANIN LEVELS DRIVE DOPAMINERGIC CELL VULNERABILITY IN PIGMENTED NON-HUMAN PRIMATES

Although a differential vulnerability of dopaminergic neurons to degeneration based on their specific location within the dorsal and ventral tiers of the substantia nigra pars compacta (SNcD and SNcV, respectively) has long been postulated, the underlying mechanisms sustaining these tier-specific differences remain poorly understood. Here, upon inducing a viral-mediated enhancement of neuromelanin (NMel) accumulation within dopaminergic neurons in non-human primates, the distribution of Lewy body-like inclusions (LBs) was analyzed within identified SNcD and SNcV neurons, together with their intracellular NMel levels. Results showed that the vast majority of intracytoplasmic inclusions were found in SNcV neurons, and indeed correlated to higher pigmentation levels. By contrast, only very few LBs were found in calbindin-positive neurons of the SNcD, which in parallel exhibited very low levels of NMel accumulation. These results postulate an additive effect made of a tier-specific location of LB burden together with high pigmentation levels as synergistic drivers sustaining the preferential vulnerability of SNcV dopaminergic neurons. Moreover, the evidence obtained here supported the conclusion that NMel accumulation beyond a given threshold triggers the aggregation of endogenous α-Syn into LBs; therefore, approaches intended to reduce pigmentation levels in SNcV neurons would likely exert a neuroprotective effect by preventing the subsequent aggregation of α-Syn.

Authors

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Reference number: T02-39

USE OF VIRAL VECTORS CODING FOR THE HUMAN TYROSINASE GENE TO INDUCE PIGMENTATION OF DOPAMINERGIC NEURONS IN MACAQUES. COMPARISON BETWEEN PUTAMINAL AND NIGRAL VIRAL DELIVERIES

The lack of adequate animal models of Parkinson's disease (PD) currently represents the main barrier for pre-clinical testing of novel therapeutic candidates. The recent introduction of a novel generation of rodent and non-human primate (NHPs) models based on the increased accumulation of neuromelanin (NMel) has gained increased appeal. These models reproduced the main neuropathological hallmarks that typically characterize human PD with unprecedented accuracy. Here, unilateral deliveries of adeno-associated viral vectors encoding the human tyrosinase gene (AAV9-hTyr) were performed into the substantia nigra pars compacta (SNpc) or the putamen in an attempt to induce a progressive pigmentation of SNpc neurons. Although both strategies managed to reproduce key PD signatures such as (i) ongoing pigmentation, (ii) Lewy body-like intracytoplasmic aggregates, (iii) time-dependent nigrostriatal damage, and (iv) pro-inflammatory scenario, viral vector deliveries into the SNpc resulted in a more severe nigrostriatal lesion and within shorter follow-up times. Compared with control

specimens, up to 62% of dopaminergic cell loss was found upon delivery of AAV9-hTyr into the SNpc after four months in-life, whereas only an 18.5% of mean neuronal degeneration was observed following putaminal injections four and eight months post-viral administration. Similar trajectories were found when considering loss of nigrostriatal terminals, resulting in 72% loss of TH+ terminals when targeting the SNpc, compared to 15% nigrostriatal terminal damage in animals where AAV9-hTyr was delivered into the putamen. In summary, targeting the SNpc led to a faster progression and more severe nigrostriatal damage, whereas a slower and milder neurodegenerative phenotype resulted from putaminal injections. Although the development of ideal NHP models of prodromal PD is extremely challenging, those appointed here could be regarded as good choices for end-stage pre-clinical testing of disease-modifying candidates.

Authors

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Reference number: T02-40

AGE-DEPENDENT PATTERNS OF NEUROMELANIN ACCUMULATION IN NAÏVE MACAQUES REVEAL NEW SIGNATURES UNDERLYING SELECTIVE NEURONAL VULNERABILITY IN PARKINSON'S DISEASE

The role of neuromelanin (NMel) in the pathophysiology of Parkinson's disease (PD) has recently gained increased interest following the introduction of novel pigmented animal models of PD (rodents and macaques) that mimicked the known neuropathological signatures that typically characterize this disorder with unprecedented accuracy. Here, the age-dependent physiological NMel accumulation in naïve macaques was investigated in macaques aged between 1 and 26 years. The conducted analyses focused on brain nuclei closely related to PD progression, such as the substantia nigra (SNpc), locus coeruleus (LC), and dorsal motor nucleus of the vagus (DMNX). Our results show that NMel accumulation remained below detection levels at 5 years of age. Pigmented neurons were first detected at 8 years of age, and their number increased until reaching a plateau at 11 years in the SNpc and LC, and remained unchanged at 26 years. By contrast, in the DMNX this plateau was reached earlier (8 years), was maintained up to 19 years, and then followed a gradual decline. In parallel, a time-dependent pattern regarding intracellular pigmentation levels was observed . In the SNpc, the accumulation of NMel gradually increased over time, reaching the highest levels at 26 years, a pattern not observed in LC neurons, where pigmentation levels remained stable over time. Regarding the DMNX, although a reduction in the number of pigmented neurons was found beyond 19 years of age, those that remained pigmented are showing a very high intracellular concentration of NMel. Consistent with previous reports, dopaminergic SNpc neurons showed the highest number of pigmented neurons and those more heavily neuromelanized, in keeping with the known vulnerability of these neurons within the context of PD.

Authors

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Reference number: T02-41

EXPLORING A NEUROPROTECTIVE ROLE FOR THE TRANSCRIPTION FACTOR REST IN PARKINSON'S DISEASE IN VIVO

The expression of the Repressor Element 1-Silencing Transcription (REST) factor is induced in the aging human brain, and it has been shown to repress cell death and promote cellular stress response in healthy aging. On the other hand, REST deletion was shown to lead to age-related neuronal loss in a conditional mouse knock-out model. In neurodegenerative pathologies, such as Dementia with Lewy Bodies (DLB), REST is lost from the nucleus; moreover, we have reported dysregulation of REST and its target genes in Parkinson's Disease (PD) models. A recent study suggests that the recruitment of REST to Lewy Bodies may prevent its neuroprotective functions in PD. This evidence highlights REST as an interesting target for PD treatment, promoting the repression of cell death, the reduction of oxidative stress and supporting mitochondrial health, which are dysfunctional in PD. The aim of this study was to confirm or discard REST as a target for PD in human SNCA-overexpressing (SNCA-OVX) mice and in the intracranial PFF (alpha-synuclein Pre-Formed Fibrils) injection model. REST overexpression was achieved through viral injection, and its effects on dopaminergic cell counts, protein aggregation, mitochondrial function, as well as on motor and cognitive performance were investigated. Collectively, our data showed a restorative effect in alpha-synuclein pathology and mitochondrial health by REST on the neurons within the Substantia Nigra pars Compacta (SNc) and the Ventral Tegmental Area (VTA). Interestingly, this was accompanied by a reduction in SNc neurons and subtle motor defects. In the SNCA-OVX animals, this was also accompanied by the rescue of anxiety phenotypes and the neuronal population of the VTA. Together our interpretation of the data is that high levels of REST, such as the ones perhaps lost in the lost population of the SNc, are toxic, while low levels of REST can be neuroprotective.

Authors

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Reference number: T02-42

DYNAMIC FTO SIGNALING REGULATES SYNAPTIC PLASTICITY AND NEURONAL FUNCTION

Synaptic plasticity, the cellular basis of learning and memory, requires precise regulation of gene expression. Among epitranscriptomic mechanisms, N6-methyladenosine (m6A) RNA modification has emerged as a key modulator of neuronal function. The fat mass and obesity-associated protein (FTO), an m6A demethylase, dynamically regulates RNA methylation, although its role in synaptic plasticity remains poorly understood.
Here, we investigated the impact of FTO on neuronal signaling, structure, and synaptic function. We found that endogenous FTO is predominantly nuclear, although its limited synaptic presence suggests a specialized role in synaptic regulation. Using different viral vectors, we further demonstrated that FTO localization critically depends on expression dynamics. Recombinant FTO levels correlated with neuronal activity and c-Fos

expression, and FTO underwent rapid nucleocytoplasmic shuttling following manipulation of key plasticity-related signaling pathways, including NMDAR and CaMKII. Functionally, altered FTO expression profoundly affected synaptic integrity and plasticity. Mislocalized FTO induced significant changes in dendritic spine organization, while multi-omics analyses revealed selective remodeling of synaptic signaling pathways, including the AKT/GSK3β axis. Additionally, electrophysiological analyses demonstrated that both FTO overexpression and silencing impair synaptic plasticity, indicating that balanced FTO activity is required for proper neuronal function.
Together, these findings identify FTO as a key epitranscriptomic regulator of synaptic plasticity, where both its expression levels and subcellular localization are critical for maintaining synaptic integrity and neuronal function.

Authors

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Reference number: T02-43

INTRA-ARTERIAL DELIVERY OF HUMAN DENTAL PULP STEM CELLS PROMOTES NEUROVASCULAR REPAIR AND FUNCTIONAL RECOVERY AFTER ISCHEMIC STROKE

Ischemic stroke (IS) induces extensive neurovascular damage characterized by blood–brain barrier (BBB) disruption, vascular degeneration, and sustained neuroinflammation, leading to long-term functional deficits. While reperfusion therapies restore cerebral blood flow, effective strategies to promote vascular repair and modulate post-ischemic inflammation remain limited. Human Dental Pulp Stem Cells (hDPSCs) are an accessible stem cell source with pro-angiogenic and immunomodulatory properties, making them promising candidates for neurovascular repair.
Here, we investigated the effects of intra-arterial administration of hDPSCs in a rat model of IS. Sprague–Dawley rats were subjected to 75 minutes of middle cerebral artery occlusion (MCAO) and received intracarotid injections of hDPSCs one hour after occlusion. Neurological function was assessed longitudinally, and animals were analyzed at 14 days post-MCAO.
Histological analyses confirmed the presence of grafted hDPSCs within infarct core and peri-infarct regions. Immunohistochemical analyses using human-specific markers (STEM121, hCD31) revealed grafted hDPSCs associated with lectin-positive vascular structures, suggesting their contribution to perfused vascular networks. Interestingly, hDPSC-treated animals exhibited significantly improved neurological recovery, accompanied by reduced infarct volume as assessed by Cresyl Violet staining. Assessment of BBB integrity using Evans Blue extravasation revealed reduced vascular leakage in hDPSC-treated animals. Importantly, analysis of the inflammatory response revealed a reduction in $CD11b^{+}$ cells within the infarcted tissue of hDPSC-treated animals, suggesting attenuation of post-ischemic neuroinflammation. Analyses of endogenous neurogenesis and neural progenitor recruitment are ongoing.
Together, these data demonstrate that intra-arterial delivery of hDPSCs promotes functional recovery through combined vascular repair, BBB stabilization, and modulation of the inflammatory response after IS.

Authors
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Reference number: T02-44

TARGETED 3´-END RNA SEQUENCING UNCOVERS CRYPTIC POLYADENYLATION IN HUNTINGTON'S DISEASE LINKED TO SOMATIC INSTABILITY AND CAG REPEAT PURITY

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by expanded CAG repeats in the first exon of the HTT gene, which encodes for huntingtin (HTT) protein. Full-penetrance is established at 40 repeats, but beyond, somatic repeat instability in the brain and CAG repeat purity modulate disease onset and severity. Previous studies have described that expanded repeats induce the incomplete splicing of HTT intron 1 to express the most pathogenic HTT isoform, known as HTT1a. Yet, the lack of a robust and sensitive method to evaluate HTT RNA-misprocessing has limited our understanding of HTT1a expression in HD pathophysiology.
Here we describe a targeted RNA sequencing approach, known as 3´-end targeted RNA sequencing or 3TRS, to simultaneously quantify multiple HTT transcripts generated by canonical and cryptic polyadenylation in several HD models. We show that activation of HTT cryptic polyadenylation is highly selective and requires long and uninterrupted CAG repeat expansions. In HD knock-in mice and human postmortem brain, cryptic HTT expression strongly correlates with brain-specific somatic repeat instability, supporting a model where ultralong and unstable CAG repeats drive toxicity by activating HTT RNA-misprocessing. Overall, 3TRS provides a robust framework to investigate HTT1a biogenesis and expression and to evaluate HTT-lowering therapeutic strategies.

Authors
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Reference number: T02-45

ANKRD55 IS ASSOCIATED WITH DIVERSE BIOMOLECULAR CONDENSATES IN IMHUM CELLS

Introduction Genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) linked to multiple sclerosis (MS) that influence the expression of ankyrin repeat domain-containing protein 55 (ANKRD55). Despite its strong genetic association with MS and other autoimmune diseases, the cellular function of ANKRD55 remains poorly understood. To explore how ANKRD55 may participate in neuroinflammatory mechanisms, we investigated its cytosolic and nuclear interactomes in the human microglial cell line IMhu-M.
Methods IMhu-M cells were transfected with synthetic ANKRD55 RNA in conjunction with nanoparticles. ANKRD55 interactomes were determined by affinity purification coupled to mass spectrometry (AP-MS) and analyzed bioinformatically. Selected candidates were validated by confocal immunofluorescence microscopy, proximity

ligation assay (PLA), and visible immunoprecipitation (VIP) assays to assess subcellular localization and direct molecular interactions.
Results ANKRD55 associated with distinct protein complexes localized to multiple biomolecular condensates. The cytosolic interactome revealed eight components of the intraflagellar transport complex B (IFT-B), with the IFT46-IFT56 heterodimer confirmed as a direct ANKRD55-binding module by VIP. Although IMhu-M cells lack primary cilia, both ANKRD55 and IFT-B proteins were enriched at the centrosome shown by confocal imaging. Moreover, ANKRD55 was present in additional membraneless condensates such as cytoplasmic P bodies and nuclear speckles. Within the nuclear fraction, the splicing factor RBM39 (RNA Binding Motif Protein 39) emerged as a major ANKRD55 interactor, as confirmed by confocal microscopy and PLA.
Conclusion Our findings demonstrate that ANKRD55 forms an IFT-B-like complex at the centrosome, independent of ciliogenesis. The interactions of ANKRD55 with several proteins, combined with its localization to centrosomes, P-bodies, and nuclear speckles, indicate that it participates in diverse membraneless organelles. Altogether, the results suggest that ANKRD55 functions as a modular and context-dependent regulator, integrating into multiprotein assemblies and biomolecular condensates to coordinate cytoskeletal organization, RNA regulation, and early differentiation processes.

Authors
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Reference number: T02-46

VACUOLAR PROTEIN SORTING-ASSOCIATED PROTEIN 4A VALIDATION AS A PARKIN SUBSTRATE AND ITS CHARACTERIZATION

Parkinson's disease (PD) is a common, chronic, and incurable neurodegenerative disorder of the central nervous system characterized by the progressive loss of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies (LBs), leading to motor and non-motor symptoms. While most PD cases are sporadic and manifest after age 50, mutations in PARK2 cause early-onset PD [1,2]. PARK2 encodes Parkin, an E3 ubiquitin ligase that post-translationally modifies proteins by covalently attaching ubiquitin to lysine residues. Identifying Parkin substrates and elucidating the functional consequences of their ubiquitination is essential to understand early-onset PD pathogenesis [3]. A previous proteomic study in Drosophila melanogaster neurons identified 35 candidate Parkin substrates. Among them, vacuolar protein sorting-associated protein 4A (VPS4A), a regulator of intracellular protein trafficking, emerged as a putative candidate [4,5]. Notably, VPS4A has also been detected in the core of LBs, suggesting a potential role in their formation. Here, we investigated whether VPS4A is a Parkin substrate in human HEK293T cells using biochemical and mass spectrometry-based approaches. GFP pull-down followed by immunoblot analysis revealed increased ubiquitination of VPS4A upon overexpression of wild-type (WT) Parkin compared to a catalytically inactive variant, supporting VPS4A as a Parkin substrate. Quantification of VPS4A::GFP levels showed an inverse correlation between ubiquitination and total protein abundance, indicating that Parkin-mediated ubiquitination promotes VPS4A degradation. Mass spectrometry analysis confirmed enhanced VPS4A ubiquitination in

cells expressing WT Parkin relative to the inactive variant. Site-specific analysis identified lysine residues K210 and K325 as preferentially ubiquitinated in the presence of WT Parkin. Additionally, increased formation of K6-, K11-, and K48-linked ubiquitin chains was detected under these conditions. Altogether, our results validate VPS4A as a novel Parkin substrate and provide proteomic insight into ubiquitin chain architecture associated with Parkin activity.

Authors

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Reference number: T02-47

ASTROCYTIC MORPHOLOGY IN THE MEDIAL HABENULA: SEX DIFFERENCES AND MODULATORY FACTORS

The medial habenula (MHb) is a subthalamic structure involved in aversive processing and emotional regulation, notable for its marked cellular heterogeneity and high astrocyte density. This cellular composition suggests that astrocytes may play an important role in MHb structure and plasticity, potentially contributing to the regulation of emotional states.

The aim of this study is to characterize sex-dependent astrocytic morphology in the MHb and determine how it is modulated by peripheral alterations and direct central manipulations. At the peripheral level, a high-fat diet (HFD) was used as a model of metabolic stress, and systemic lipopolysaccharide (LPS) administration was used to induce a peripheral inflammatory challenge. At the central level, astrocyte-specific deletion of the cannabinoid type 1 receptor (CB1) was combined with LPS administration to evaluate the role of CB1 signaling in astrocytic responses to inflammation. Additionally, a chemogenetic approach using Gi-DREADDs under the GFAP promoter allowed selective modulation of astrocytic intracellular signaling independently of peripheral influences.

Preliminary results indicate sex-dependent morphological differences in MHb astrocytes across all these experimental conditions, supporting the idea that MHb astrocytes are sensitive to both peripheral and central disturbances and may represent a key cellular substrate linking body-brain interactions with emotional regulation.

Authors

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Reference number: T02-48

PGRN AS A KEY REGULATOR IN THE INTERACTION BETWEEN RIBOSOME AND LYSOSOME UNDER METABOLIC STRESS IN NEURODEGENERATION

Objectives: Mutations in the GRN gene, encoding Progranulin (PGRN), are a major cause of Frontotemporal Dementia (FTD). PGRN deficiency impairs lysosomal function, leading to the toxic accumulation of substrates like TDP-43, a central hallmark of the FTD-ALS

spectrum. While Ribosomal Quality Control (RQC) systems manage oxidative stress , their collapse drives the cytoplasmic accumulation of non-degraded ribosomal subunits and ribonucleoproteins. We hypothesize that defective ribophagy in PGRN-deficient cells prevents the proper resolution of this ribostatic stress. Therefore, the primary objectives of this study are to determine whether PGRN participates in the dynamics of stress-induced stress granules and to assess if its deficiency impairs proteostatic recovery and ribophagy following acute stress.
Material and methods: To investigate this, we utilized several human cellular models, including neuroblastoma lines (wild-type and GRN KO), an inducible U2OS line, and iPSC-derived glutamatergic neurons. Cells were exposed to acute oxidative stress to evaluate stress granule dynamics and the recovery of protein synthesis. Additionally, we employed an RPS3-keima reporter assay to directly monitor ribophagic flux.
Results: Following acute intracellular stress, PGRN was observed to co-localize within cytoplasmic stress granules alongside ribosomal components and TDP-43. Furthermore, GRN KO cells exhibited significantly impaired and delayed protein translation recovery upon stress alleviation. This was accompanied by an abnormal, persistent accumulation of specific ribosomal proteins without a corresponding increase in their transcription, indicating a clearance defect. Concurrently, the RPS3-keima assay revealed that PGRN deficiency directly impairs ribophagy, hindering the degradation of damaged ribosomes.
Conclusion: PGRN plays a crucial physical and functional role in managing ribostatic stress. Its deficiency results in defective ribophagy and impaired ribosomal subunit clearance, which stalls translational recovery. This failure to adequately restore cellular proteostasis likely prolongs ribostatic stress, creating a permissive environment for TDP-43 misfolding and the accumulation of pathogenic inclusions characteristic of GRN-associated neurodegeneration.

Authors

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Reference number: T02-49

TURNING THE TIDE IN GLIOBLASTOMA: A NEW THERAPEUTIC APPROACH EXPLOITING NAE1 OVEREXPRESSION VIA SELECTIVE NEDDYLATION HYPER-ACTIVATION

Glioblastoma multiforme (GBM) exhibits a distinct molecular profile characterized by the upregulation of Neddylation Activating Enzyme E1 subunit 1 (NAE1), as revealed by bioinformatic analysis of clinical databases. Paradoxically, this overexpression does not always correlate with canonical increases in total protein neddylation or the degradation of classical substrates like p27, suggesting a "primed" but latent state of the pathway. Leveraging this clinical feature, we investigate IBA0224, a novel agonist of the Neddylation pathway. Our main objective is to correlate clinical bioinformatic data of NAE1 expression with the pharmacological response to IBA0224 in U251 human glioma cells and to validate its mechanism through NAE inhibition, proposing a new therapeutic strategy based on forcing the hyper-activation of this machinery to induce proteotoxic collapse in GBM.

With this aim, bioinformatic expression profiling of NAE1 and different Neddylation pathway components was performed using public GBM datasets (TCGA/CGGA). Bioinformatic analysis confirmed that NAE1 and CUL1 are significantly overexpressed in GBM compared to healthy brain tissue (GTEx), with high expression strictly correlating with dismal patient survival ($p < 0.05$). Spatial analysis (Ivy GAP) revealed a significant enrichment of the NAE1/UBA3 axis specifically in the pseudopalisading cells (PAN) and perinecrotic zones (PNZ). Crucially, we identified NAE1 as a key driver of the “Glioma Stem Cell Identity” phenotype, showing a powerful correlation with SOX2 (R=0.63) and Nestin (R=0.28). Furthermore, NAE1 expression was associated with p53 (R=0.47) and Ki-67, suggesting its role in sustaining rapid proliferation under oncogenic stress. Importantly, the lack of correlation with the astrocytic marker GFAP highlights a potential therapeutic window. Besides, in vitro validation was conducted in U251 cells treated with different concentrations (100 nM, 500 nM, and 1µM) at different time points (24h, 48h, and 72h) with the neddylation agonist, IBA0224. Experimentally, IBA0224 effectively exploited this pathway, inducing a delayed but massive cytotoxic response (48-72h). This effect was accompanied by the hyper-neddylation of specific protein targets. Crucially, co-treatment with a sub-lethal concentration of MLN4924, a Neddylation inhibitor in different Phase 2/3 clinical trials for several cancers, completely abolished the IBA0224-induced phenotype, confirming that the overexpressed NAE1 machinery is the essential vehicle for IBA0224-mediated lethality. Our study bridges clinical bioinformatic observations with experimental pharmacology. We demonstrate that the elevated levels of NAE1 in GBM, while not always linked to canonical pathway flux, constitute a therapeutic window that can be exploited by agonists like IBA0224. This "activation-based" strategy represents a promising alternative to traditional inhibition in neuro-oncology.
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Reference number: T02-50

COMPREHENSIVE ANALYSIS OF RNA MISPROCESSING UNCOVERS THERAPEUTIC VULNERABILITIES IN GLIOBLASTOMA

Glioblastoma (GBM) is the most frequent and most aggressive brain tumor, with a devastating prognosis and is directly categorized as high-grade glioma (HGG). Its aggressiveness is closely associated with a strong therapy resistance which arises from a small population of dormant but highly tumorigenic self-renewing glioma stem cells (GSCs). RNA-processing is a tightly regulated molecular mechanism that includes 5’mRNA capping, splicing and 3’end processing. Misregulation of RNA-processing is a hallmark of cancer that contributes to tumor initiation and progression. The main objective of this project is to uncover RNA-misprocessing signature of GBM that can be exploited to develop new therapeutic approaches.

First, we obtained RNA sequencing data of high-grade GBM (n=49) and low-grade gliomas (LGG) astrocytoma (n=34) and oligodendroglioma (n=39) samples from Chinese Glioma Genome Atlas (CGGA) database. Gene expression and alternative splicing (AS) were analysed using Salmon and Vast-tools pipelines, respectively. Our analysis demonstrated that GBM not only separates from LGGs by gene expression, but also by splicing profile. We detected many dysregulated AS events comparing GBM versus LGGs. However, there were few dysregulated AS events when comparing LGGs between them, confirming that RNA-misprocessing is a molecular hallmark associated with higher tumor grade.
Based on this analysis, we have identified a specific AS signature associated with GBM aggressiveness that constitutes an additional layer of molecular regulation of genes involved in tumor malignancy, including cytoskeletal organization and cell-junction pathways. We experimentally validated the dysregulation of several AS events by RT-PCR in tumor samples of glioma patients deposited in Basque Biobank. In addition, by selectively modulating the splicing of target events using antisense oligonucleotides (ASOs) in patient-derived GSCs cell lines, we studied the contribution of individual splicing events to GSCs fitness and evaluated the impact of splicing modulation on key cellular processes, such as cell viability, proliferation and migration. Although splicing modulation results in heterogeneous functional outcomes across targeted events, highlighting the differential impact that a singular AS event has in malignant phenotype, we identified a target event with potential to impair tumor cell survival and proliferation, positioning its splicing modulation as a promising therapeutic avenue against GBM.

Authors
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Reference number: T02-51

LIMITED IMPACT OF WNT/B-CATENIN ACTIVATION ON THE GROWTH OF HDPSC-DERIVED NEURAL SPHEROIDS

Neurodegenerative diseases represent a major public health burden worldwide. In recent years, brain organoids have been emerged as a promising platform to model the disease disorders and accelerate drug discovery. However, current organoid models present important limitations, including impaired growth kinetics, absence of vascularization and ethical constraints associated with pluripotent stem cell use. Human dental pulp stem cells (hDPSCs) are multipotent ectomesenchymal stem cells of neural crest origin and represent an attractive alternative cell source. When exposed to an established hESC-based brain organoid protocol, we observed that hDPSCs display reduced proliferative capacity, which impacts spheroid growth dynamics and limits long-term expansion. Given the central role of the Wnt/ -catenin pathway in regulating proliferation, migration and cell survival, we hypothesized that its activation could enhance hDPSC-derived neural spheroid growth.
The objective of this study was to evaluate wether activation of the Wnt/β-catenin pathway improves the growth of hDPSC-based neural spheroids. Third molars from donors aged 18-40 years were collected, and hDPSCs were isolated from the pulp chamber. Following expansion under standard culture conditions (DMEM supplemented with 10% FBS), cells were transferred to a modified hESC medium supplemented with

either Afamin/Wnt3a-conditioned medium or a recombinant human Wnt3a surrogate to promote proliferation. Spheroid diameter was monitored by daily imaging.
Our results demonstrate that neither Afamin/Wnt3a-conditioned medium nor a Wnt3a surrogate significantly enhanced spheroid growth in modified hESC medium. Although Wnt activation did not increase spheroid diameter under any condition, a reduced rate of size decline over days in vitro was observed in Wnt3a-treated samples compared to untreated controls. This suggests that Wnt3a stimulation, whether delivered via Afamin-associated ligand or surrogate, may activate cellular mechanisms that help preserve spheroid integrity during early stages. Therefore, while insufficient to directly promote spheroid expansion, Wnt signaling could support the maintenance of hDPSC-derived aggregates during the initial phases of neural organoid formation, potentially improving their competence for subsequent neuroinduction steps.

Authors
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Reference number: T02-52

A 3D MODEL OF HUMAN LOCUS COERULEUS TO STUDY THE EFFECT OF E46K A-SYNUCLEIN MUTATION.

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease. Although motor symptoms such as bradykinesia, tremor and rigidity are primarily associated with the degeneration and death of dopaminergic neurons in the Substantia nigra, non-motor symptoms such as behavioral changes or sleep disturbances, that frequently precede the onset of motor symptoms, may be related to dysfunction of noradrenergic pathways arising from neurons in the Locus coeruleus (LC).
Certain forms of PD are associated with genetic mutations, such as the E46K mutation in α-synuclein (SNCA), a rare mutation identified in a Basque family with a history of parkinsonism. To investigate the effects of this mutation on α-synuclein, we are using three-dimensional cellular models, brain organoids, derived from human induced pluripotent stem cells (iPSCs)— obtained from patients carrying the E46K mutation. Our first aim was to generate LC organoids by recreating the structure, physiology and overall in vivo development. We evaluated the inductive effect of two protocols. As controls, we used isogenic lines with the reverted mutation, as well as organoids derived from a healthy control.
We first sought to characterize whether the organoids had successfully differentiated into the LC. We analyzed by immunofluorescence and confocal microscopy the expression of phenotypic markers and confirmed the presence of noradrenergic neurons by the coexpression of dopamine-β-hydroxylase (DBH) and the transcription factor PHOX2B in organoids derived from all the cell lines.
We subsequently studied both α-synuclein and phosphorylated-α-synuclein at Ser129 to explore the differences between mutant and healthy lines. To compare and quantify the protein levels of α-synuclein, we used western blot with a microfluidic platform (ProteinSimple's Jess™), using both polyclonal and monoclonal α-synuclein antibodies, with the former enabling the detection of oligomeric forms. As an additional control, we used protein extract from human brain. This analysis was complemented with

immunofluorescence analysis, as the Jess™ platform allows direct protein quantification, but does not provide information on protein localization.
Our preliminary results show that in this platform we can consistently detect monomeric and oligomeric forms of α-synuclein. Quantification of experiments run at 60, 90 and 120 div showed no significant differences in the total content of α-synuclein between control and mutated organoids at the examined timepoints. Due to the presence of other neurons in the organoids, we will need to perform a more detailed analysis of the cellular content of α-synuclein in noradrenergic neurons to rule out an effect of the mutation using immunofluorescence staining.
In conclusion, we have set up a three-dimensional personalized model of the human Locus coeruleus, that allows the investigation of the pathogenic processes in early stages of PD related to E46K SNCA mutation such as expression and phosphorylation of α-synuclein, within a physiologically relevant microenvironment.

Authors
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Reference number: T02-53

MODELLING PARKINSON'S DISEASE WITH IPSC-DERIVED MIDBRAIN ORGANOIDS.

Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra (SN). Although most cases occur sporadically, some familial forms have been linked to specific mutations, such as the E46K mutation in the α-synuclein gene, described in a Basque family with a history of parkinsonism and Lewy body dementia. To further our understanding of the disease, we developed a three-dimensional (3D) cell model based on this mutation.
First, we focused on optimizing mesencephalic differentiation in 3D of induced pluripotent stem cell (iPSC) lines carrying the E46K mutation, as well as isogenic control lines in which the mutation has been corrected, using morphogens important for embryonic midbrain specification including WNT1, FGF8 and SHH. We studied eight different iPSC cell lines, including E46K lines, isogenic corrected controls, patient lines, and a control cell line.
We compared the effect of two different conditions (0.8 CHIR and 1.6 CHIR) analyzing the presence of TH positive neurons at 60 and 90 days using immunofluorescence and confocal microscopy in mesencephalic organoids. In addition, calbindin and SOX6 were used to evaluate TH neuronal populations with different vulnerability present in the organoids. We also examined the expression of α-synuclein and phosphorylated α-synuclein, as well as p62, for their involvement in disease pathology and quantified their levels using Simple Western Jess™.
Our results showed a successful differentiation towards dopaminergic neurons under both inductive conditions independently of the mutation status.TH quantification did not reveal major differences between the 0.8 and 1.6 CHIR conditions, suggesting a similar differentiation efficiency. Intriguingly, in the isogenic pairs, mutant organoids did show an increase in α-synuclein only under low CHIR conditions, while under high CHIR conditions the control lines showed a higher content at both time points.
In summary, these results support the use of IPSC-derived midbrain organoids as a promising 3D model for studying cellular and molecular mechanisms involved in PD.

Futhermore, this model may contribute to a better understanding of processes associated with dopaminergic neurodegeneration and alterations related to α-synuclein in a more physiological context.

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Reference number: T02-54

PRE-EXPANSION ENHANCES HDPSC NEURAL YIELD WITHOUT COMPROMISING NEURAL DIFFERENTIATION

Human Dental Pulp Stem Cells (hDPSCs) – adult stem cells residing in the dental pulp chamber of deciduous teeth – represent a promising alternative for neuronal differentiation, due to their neuroectodermal origin, limited bioethical constraints and ease of extraction and culture. We previously developed an ex vivo neural differentiation protocol independent of genetic modification that enables these cells to express voltage dependent channels and neurotransmitter receptors, as well as to display electrophysiological excitability [1].

With the aim of increasing the yield of differentiation, this study compared our established neural differentiation protocol, in which freshly isolated cells are directly cultured forming dentospheres in neural medium, with a modified approach including a pre-expansion step under standard adherent conditions. Both groups were subjected to identical neural differentiation conditions, including retinoic acid (RA) and potassium chloride (KCl) stimulation protocols adapted from Pardo-Rodriguez et al [1]. After 21DIV, neural phenotype was assessed by qPCR and immunofluorescence (IF).

Our results show that the initial pre-expansion step maintains the stem cell phenotype Nestin ($p = 0.5$), with a three-fold increase in stem cell number available for differentiation. After completion of the differentiation protocol, no significant differences were observed between conditions in the expression of neural markers assessed by qPCR, including, and MAP2 ($p = 0.75$) and GFAP ($p > 0.999$). Similarly, IF analysis of Nestin ($p = 0.75$) and GFAP ($p = 0.5$) did not reveal significant differences.

In conclusion, pre-expansion of hDPSCs cultures in adherent conditions is a valid alternative to enhance the efficiency of the neural differentiation protocol without significantly compromising neural differentiation.

[1] Pardo-Rodríguez B, Baraibar AM, Manero-Roig I, Luzuriaga J, Salvador-Moya J, Polo Y, Basanta-Torres R, Unda F, Mato S, Ibarretxe G, Pineda JR. Functional differentiation of human dental pulp stem cells into neuron-like cells exhibiting electrophysiological activity. Stem Cell Res Ther. 2025 Jan 23;16(1):10. doi: 10.1186/s13287-025-04134-7.

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Reference number: T02-55

PKC-DEPENDENT MYRF DYSREGULATION DRIVES OLIGODENDROCYTE AND SPATIAL MEMORY DEFECTS IN ALZHEIMER'S DISEASE

Oligodendrocyte and myelin alterations are increasingly recognized as contributors to Alzheimer's disease (AD) pathophysiology and cognitive decline. We previously showed that amyloid-β (Aβ) promotes oligodendrocyte differentiation both in vitro and in vivo;

however, the molecular mechanisms underlying oligodendrocyte dysfunction in AD remain poorly understood. Myelin regulatory factor (MYRF), a transcription factor essential for oligodendrocyte maturation and myelin maintenance, represents a strong candidate, yet it is unknown whether its regulation is altered in the disease and how such changes may contribute to glial pathology.
Here, we found that 3xTg-AD mice display aberrantly promoted oligodendrocyte maturation dynamics and reduced oligodendroglial populations in the dentate gyrus, together with increased MYRF protein expression. MYRF upregulation was also detected in Aβ-injected wild-type mice, hippocampal samples from AD patients with high Aβ burden, and Aβ-treated oligodendrocytes, where sustained MYRF overexpression impaired cell viability. Mechanistically, Aβ induced post-translational dysregulation of MYRF by altering GSK3/PKC-dependent pathways involved in MYRF degradation, thereby prolonging the half-life of its nuclear form. Importantly, intracerebroventricular administration of the PKC inhibitor Gö6983 reduced MYRF levels in 3xTg-AD mice, restored oligodendrocyte and myelin integrity, and improved hippocampal-dependent spatial learning and memory in the Barnes Maze.
Together, these findings identify MYRF dysregulation as a novel feature of AD and establish a mechanistic link between Aβ/PKC signalling and oligodendrocyte pathology. Moreover, these results support PKC inhibition as a potential therapeutic strategy for AD.

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Reference number: T02-56

TRANSCRIPTOMIC IDENTIFICATION OF IRF5 AS MARKER OF MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system in which microglia regulate the balance between tissue damage and remyelination. IRF5, classically characterised as a pro-inflammatory TLR/MyD88 effector, has recently been implicated in myelin debris processing and microglial cholesterol homeostasis, with reparative roles in MS lesion resolution. However, its expression across microglial states and its function along disease progression remain uncharacterised. To address this, we integrated five publicly available human brain sc/snRNA-seq cohorts, comprising 239,205 nuclei from healthy controls and MS patients at different lesion stages. Sub-clustering of the myeloid compartment (11,433 nuclei) resolved seven states: homeostatic (HM1–HM3), DAM–LDAM, hypoxic–glycolytic, proteostatic stress, and perivascular/meningeal macrophages. The IRF5^{+} signature was transcriptionally orthogonal to canonical reactive microglial states in MS, sharing no core markers with DAM, MGnD, MIMS-foamy or MIMS-iron, nor with any of the clusters defined in our integrated dataset. Nevertheless, IRF5^{+} microglia were enriched in active lesions compared with normal-appearing white matter. Differential expression analysis of IRF5^{+} versus IRF5^{-} cells (MAST) revealed that IRF5^{+} microglia upregulate Rho-GTPase signalling regulators (VAV1, ARHGAP25, ADGRG1, DOCK10) alongside canonical innate immune effectors (TLR1, NAIP, BLNK) — a programme consistent with enhanced

phagocytic and motile competence. Consistent with this, transcriptomic profiling of Irf5$^{-/-}$ microglia in our previous work revealed dysregulation of the same Rho-GTPase axis, supporting a conserved role for IRF5 in coordinating cytoskeletal and immune programmes. To validate these findings in a human cellular context, iPSC-derived microglia (iMGLs) were generated using optimised protocols and confirmed by myeloid morphology and Iba1 immunoreactivity. iMGLs were assayed using labelled myelin to determine the role of irf5 on myelin phagocytosis. Together, these findings redefine IRF5 as a regulator of a previously unrecognised cytoskeletal–phagocytic microglial state in MS and open therapeutic avenues targeting IRF5-dependent functions in lesion resolution.

Authors

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Reference number: T02-57

ANALYSIS OF HIPSC-DERIVED REACTIVE ASTROCYTES FROM MULTIPLE SCLEROSIS PATIENTS WITH A TNFRSF1A POLYMORPHISM

Multiple sclerosis (MS) is a chronic inflammatory disorder of the human central nervous system (CNS) characterized by the emergence of demyelinating lesions leading to neurodegeneration and progressive neurological disability. The pathogeny of MS is complex and involves a combination of genetic and environmental factors.
Astrocytes perform essential homeostatic functions in the CNS and acquire reactive phenotypes in the neuroinflammatory context of MS that promote disease pathogenesis through multiple mechanisms. Astroglial cells rely on metabolic reprogramming to meet increased bioenergetic demands during activation and current research lines suggest that astrocytic metabolic switch accelerates neurotoxicity in MS. However, the roles and metabolic phenotypes of astrocytes in MS have been primarily studied using rodent models and postmortem human tissues that fail to fully capture the complexity of MS pathophysiology.
This study evaluated the inflammatory and metabolic phenotype of human astrocytes in response to pro-inflammatory stimuli associated to MS pathology. Astrocytes were differentiated from human induced pluripotent stem cells derived from one healthy donor and two MS patients carrying either the TT (MSTT) or CC (MSCC) genotypes of the TNFRSF1A polymorphism rs1800693 that confers genetic risk for MS. Pro-inflammatory factors increased the expression of inflammatory genes and upregulated basal glycolysis and lactate flux in control, MSTT and MSCC astrocytes. We observed differences between control and MS conditions with regard to the expression of specific inflammatory genes such as C3, CXCL10 and MMP3. These results increase our understanding of human astrocyte reactivity and point to disease-relevant phenotypes of the rs1800693 polymorphism modulating inflammation associated to MS.
Keywords: human astrocytes, metabolism, inflammation, multiple sclerosis
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Reference number: T02-58

DEVELOPMENT OF A FLUOROMETRIC ASSAY TO QUANTIFY DAGL ACTIVITY AND ENDOCANNABINOID SIGNALING IN MOUSE SYNAPTOSOMAL MEMBRANES

Diacylglycerol lipases (DAGLs) are key serine hydrolases involved in the biosynthesis of the endocannabinoid 2-arachidonoylglycerol (2-AG), a major lipid mediator regulating synaptic transmission and neuroinflammatory processes in the brain. Despite their physiological relevance, reliable quantification of DAGL activity in native tissue remains technically challenging due to the lack of simple, sensitive, and real-time assays compatible with native membrane preparations. In this study, we implemented and optimized a fluorometric assay based on the EnzChek substrate, a quenched fluorescent triglyceride analog that becomes fluorescent upon enzymatic hydrolysis, to measure lipase activity in mouse hippocampal synaptosomal membranes.

Experimental parameters including detergent composition, substrate solubilization, protein concentration, and assay buffer were systematically optimized to maximize signal linearity and enzymatic performance. Under optimized conditions, Michaelis–Menten kinetic analysis revealed an apparent KM of 5.4 μM and a Vmax of 14.29 pmol min^{-1} μg^{-1} protein. Inhibitor profiling using selective compounds and activity curves generated across increasing concentrations enabled quantitative resolution of the contribution of individual serine hydrolases to total activity and determination of their inhibitory potencies. These analyses demonstrated that the majority of the activity in synaptosomal membranes was attributable to DAGL enzymes, predominantly DAGLα, with smaller contributions from DAGLβ and ABHD6.

Altogether, this work establishes a robust and reproducible fluorometric assay for monitoring DAGL-associated enzymatic activity in native synaptosomal membranes, providing a valuable tool for the functional study of endocannabinoid metabolism and the pharmacological dissection of serine hydrolase activity in both physiological and pathological brain tissue.

This work was supported by the Basque Government (IT1873_26), the Spanish Ministry of Science and Innovation (Grant PID2022-138266NB-I00, funded by MCIN/AEI/10.13039/501100011033 and by ERDF, “A way of making Europe”), and the EHU project EHU-N25/14.

Authors

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Reference number: T02-59

MEMBRANE CHOLESTEROL CONSTRAINS CB1 RECEPTOR ACTIVATION IN NATIVE CORTICAL SYNAPTOSOMES

The endocannabinoid system is a major neuromodulatory network involved in the regulation of synaptic transmission and neuronal activity throughout the brain. The CB1 cannabinoid receptor, which is highly enriched at presynaptic terminals, plays a central role in controlling neurotransmitter release and shaping neuronal communication. Although CB1 receptor signalling has been extensively studied, less is known about how

the membrane environment influences receptor function in native brain tissue. Given the high cholesterol content of neuronal membranes, understanding its impact on CB1 receptor signalling is essential to better define the contextual regulation of endocannabinoid signalling in the brain.
Here, we investigated the role of membrane cholesterol in modulating CB1 receptor function using rat cortical synaptosomes, a native preparation enriched in functional presynaptic terminals. Cholesterol depletion with methyl-β-cyclodextrin significantly enhanced CB1 receptor signalling, whereas cholesterol replenishment reversed this effect, indicating that membrane cholesterol normally constrains receptor activation in cortical synaptic membranes. This modulation was more pronounced for lower-efficacy cannabinoids, suggesting that membrane composition shapes the signalling properties of different cannabinoid ligands. In parallel, cholesterol depletion reduced the binding of the inverse agonist SR141716A, a ligand that preferentially recognizes inactive CB1 receptor states, supporting the idea that membrane cholesterol favours less active receptor conformations under basal conditions. Quantitative analyses confirmed that these effects were not associated with changes in receptor or G protein abundance, suggesting that reduced membrane cholesterol enhances CB1 receptor–G protein coupling rather than altering the expression of the signalling components.
Together, these findings identify membrane cholesterol as a key regulator of CB1 receptor function in native rat cortical synapses and support the idea that the local lipid environment contributes to the fine-tuning of endocannabinoid signalling in the brain.
This work was supported by the Basque Government (IT1873_26) and the Spanish Ministry of Science and Innovation (Grant PID2022-138266NB-I00, funded by MCIN/AEI/10.13039/501100011033 and by ERDF, “A way of making Europe”).
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Reference number: T02-60

CONTEXT-DEPENDENT REGULATION OF TGF-B/SMAD3 SIGNALLING IN HUMAN DENTAL PULP STEM CELLS UNDER NEUROGENIC CULTURE CONDITIONS

The Transforming Growth Factor beta (TGF-β) pathway is a key signalling mechanism involved in neural stem cell quiescence, ageing [1], and cell cycle lengthening [2]. It also exhibits a well-established dual role in cancer biology, functioning both as a tumour suppressor and as a tumour promoter depending on the cellular context and micro ambient [3]. Importantly, no tumorigenic potential has been reported for human dental pulp stem cells (hDPSCs) [4-5], and the role of TGF-β signalling in these cells remains poorly characterized. Therefore, investigating TGF-β signalling in hDPSCs cultured in DMEM and NeuroCult neurogenic medium could provide relevant insights for the optimization and safety of hDPSC-based regenerative therapies.
Our results demonstrate that hDPSCs express TGF-β receptors type I and II under both standard culture conditions (DMEM) and serum-free neurogenic conditions (NeuroCult™). Notably, increasing cell confluence was associated with reduced expression levels of both receptors. Under DMEM conditions, activation of the TGF-β

pathway was assessed by quantifying the proportion of cells exhibiting nuclear phosphorylated SMAD3 (pSMAD3). hDPSCs showed a clear activation response following stimulation with ligands TGF-β1 or TGF-β2 (10 ng/mL) after 1 hour. When cells were pretreated with the TGFBR1 inhibitor SB431542 before ligand stimulation, cells showed no activation response, displaying similar levels to unstimulated ones. In contrast, when cells were cultured in NeuroCult™ medium, higher basal levels of pSMAD3 were observed. Under these conditions, stimulation with TGF-β1 (10 ng/mL, 1 hour) resulted in only a modest and non-significant increase in pathway activation compared to baseline.

In conclusion, hDPSCs display a functional TGF-β signalling pathway whose activation is strongly conditioned by culture conditions and cell density. Neurogenic medium induces higher basal pathway activity, limiting additional responsiveness to exogenous TGF-β and highlighting its relevance for optimising hDPSC-based therapies.

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Reference number: T02-61

NUCLEOTIDE RECYCLING IMBALANCES AND MTDNA INSTABILITY: A CASCADE LEADING TO NEURONAL DEATH IN PARKINSONISM

The degeneration of dopaminergic neurons in Parkinson's disease (PD) has traditionally been attributed to mitochondrial energy failure. However, emerging evidence highlights aberrant DNA metabolism and impaired cellular recycling (autophagy) as critical pathogenic contributors. Since mitochondria contain DNA (mtDNA) essential for both oxidative phosphorylation and the regulation of autophagy, we propose that imbalances in nucleotide recycling trigger a cascade of mtDNA maintenance defects. These defects, in turn, disrupt autophagy and activate DNA damage responses, ultimately leading to neuronal death.

In this study, we focus on a specific protein essential for mtDNA integrity, whose deficiency in humans is linked to multisystemic disorders and Parkinsonism. Using Drosophila melanogaster as a model organism, we characterized the neurological impact of silencing this gene, revealing significant functional abnormalities, mitochondrial impairment, and alterations in neurotransmitter metabolism. These

findings demonstrate that our model effectively recapitulates the clinical hallmarks of the disease, providing a robust platform to explore the genetic and metabolic factors contributing to neurodegeneration and to identify potential therapeutic targets.

Authors

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Reference number: T02-62

NEW INSIGHTS INTO NEURODEGENERATIVE PROTEINOPATHY: A NOVEL THERAPEUTIC TARGET AGAINST TDP-43 SPREADING IN ALS.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons and the aggregation and spreading of protein pathology across interconnected cell types, like TDP-43. Considering increased evidence about the importance of non-autonomous mechanisms in disease propagation, we developed a Drosophila model with a conditional glial overexpression of human TDP-43 (hTDP-43), resulting in a time-dependent progressive spread of hTDP-43 to neurons. Single-cell RNA sequencing of brains highlighted novel candidate pathways underlying hTDP-43 spread. Consistently, genetic ablation of our target in the Drosophila model improved motor capacity, prolonged lifespan and halted the hTDP-43 accumulation in non-glial cells. Moreover, the mechanisms identified in Drosophila were validated in human cell models, using an experimental design that recapitulates pathological transmission between cells.Based on the results of computational structural modeling , an FDA-approved compound was identifiedas a stable binder of certain key protein structures. Experimental validation demonstrated that treatment with this agent improved locomotor performance and survival in in vivo models, while significantly attenuating the intercellular transmission of pathological protein aggregates in human cell assays. Collectively, these findings elucidate a critical mechanism for disease progression and highlight new targets for limiting proteopathic spread.

Authors

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Reference number: T02-63

TARGETING NEUROINFLAMMATION TO PRESERVE NEURONAL NETWORK ACTIVITY IN EPILEPSY

Despite decades of research, neurocentric therapeutic strategies for epilepsy have shown limited progress, while increasing evidence highlights the critical role of glial activation and neuroinflammation in epileptogenesis. Early inhibition of gliosis and neuroinflammation may help preserve normal neuronal and network function in vivo. This project investigates how blocking these processes influences the transition from physiological to pathological neuronal dynamics during epileptogenesis and whether it contributes to the preservation of GABAergic neurons. Using a well-established mouse

model of mesial temporal lobe epilepsy (MTLE), inhibition of the STAT3 signaling pathway is used to block gliosis and neuroinflammation. The effects of this intervention are assessed through three complementary approaches: (1) in vivo calcium imaging of neuronal and network activity in awake, freely moving mice using a wearable miniscope, (2) evaluation of the anti-inflammatory effects of the STAT3 inhibitor WP1066 in the hippocampus, and (3) analysis of the preservation of hippocampal GABAergic neurons. By moving beyond the traditional neurocentric view of epilepsy, this work provides new insight into the role of glia and neuroinflammation in epileptogenesis and contributes to identifying potential therapeutic strategies for this prevalent neurological disorder.

Authors

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Reference number: T02-64

BAROREFLEX-INDUCED NEUROVASCULAR COUPLING IN THE NUCLEUS OF THE SOLITARY TRACT IS BLUNTED AFTER HEART FAILURE

Heart failure (HF) is a major public health burden, and blunted baroreflex (BR) function is a defining feature of this disease. The nucleus of the solitary tract (NTS) integrates baroreceptor inputs to maintain cardiovascular homeostasis and serves as a central target of renin-angiotensin system (RAS)-driven baroreceptor dysfunction. Yet, the mechanisms that link neuronal activity in the NTS to local vascular responses, an essential process for maintaining normal neuronal signaling and functional integrity, remain poorly understood. Neurovascular coupling (NVC), the process matching neuronal activity with local cerebral blood flow (CBF), is impaired in the cortex during HF and contributes to cognitive decline. Whether similar NVC deficits occur in the NTS and contribute to blunted BR function is unknown. To test this, we developed a novel in vivo rat model that enables high-resolution two-photon imaging of NVC within the NTS during BR activation. Using baroreceptor loading (IV phenylephrine, to increase blood pressure) and unloading (IV sodium nitroprusside to reduce blood pressure), we can directly measure NTS microvascular responses, including changes in arteriole diameter and blood flow (reported as change in red blood cell (RBC) velocity) in control and myocardial infarction HF rats. Rats received a low dose (2.5 g/kg, change in mean arterial pressure (MAP) of +21.86 mmHg and change in heart rate (HR) of -4.28 bpm) and high dose (25 g/kg, change in MAP of +55.54 mmHg and change in HR of -130.6 bpm) of phenylephrine to test BR-induced NVC responses. Intravenous infusion of phenylephrine leads to dose-dependent increases in RBC velocity (28.89 4.01% vs. 51.23 8.43% increase in velocity, p=0.0398). The low dose (1.98 0.34% increase in diameter) and high dose (2.80 0.53% increase in diameter) phenylephrine led to vasodilation in control animals. Rats that have undergone HF, however, show a blunted RBC velocity change (low dose: -13.32 2.04 % change in velocity, high dose: -7.69 2.93% change in velocity, p<0.0001 compared to respective control conditions) and unveiling of a vasoconstriction (low dose: -2.08 0.42% change in diameter, high dose: -2.01 0.31% change in diameter, p<0.0001 compared to respective control conditions) to phenylephrine infusion. In contrast, BR unloading via sodium nitroprusside (25 g/kg, change in MAP of -45.80 mmHg and change in HR of +58.30 bpm) led to a vasoconstriction (-0.84 0.24% decrease in diameter) and reduction in flow (-14.86 6.58% change in velocity) that is not blunted after HF (-0.25 0.41% decrease in

diameter and -12.57 4.45% change in velocity). Our data demonstrate for the first time: (1) robust BR-evoked NVC responses, and (2) blunted BR-evoked functional hyperemia in a myocardial infarction model of HF. Future investigations will aim to (1) define the mechanisms mediating BR-evoked NVC in the NTS and (2) determine whether RAS overactivation drives impaired NVC in HF, and whether its inhibition restores normal vascular responses. By establishing NVC as a novel mechanism contributing to autonomic imbalance in HF, this research identifies new therapeutic targets to improve BR regulation.

Authors

Stern, JE and Carson KE

Reference number: T02-65

RECONNECTING IMPLANTS AS THERAPY FOR FUNCTIONAL RECOVERY OF SPINAL CORD INJURY

Our study investigates the use of electroconductive carbon nanotube (CNT) containing biomaterials as a way for restoring the functional connectivity of injured spinal cord. To do so, we use a complete spinal cord transection model, at T10 level, in Sprague–Dawley rats. The primary objective of this project is to assess if our scaffolds enhance motor recovery after severe injury.

Sprague–Dawley rats undergo a T10 spinal cord transection, followed by implantation of CNT-based materials at the lesion site. We assess their functional recovery over a six-week period using the established behavioural tests Basso, Beattie and Bresnahan (BBB) locomotor rating scale and the horizontal ladder walking test. This allows for the quantitative evaluation of hindlimb motor function, coordination, and stepping accuracy. After the 6 weeks, animals are euthanized for exhaustive tissue analysis. The spines are extracted and later analysed using magnetic resonance imaging (MRI) to verify that the lesions have been performed correctly. We exclude samples showing incomplete transections, making sure we maintain experimental consistency across the different groups.

Preliminary results show improved locomotor activity in animals implanted with CNT-containing materials compared to control groups, which display limited or no functional recovery over the same period of time. These findings suggest a potential beneficial effect of our scaffolds on motor function recovery after spinal cord injury.

Authors

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Reference number: T02-66

CELL-TYPE SPECIFIC ELECTROPHYSIOLOGICAL ALTERATIONS OF EPILEPTIFORM ACTIVITY IN THE HIPPOCAMPUS.

Epileptiform activity (EA) refers to abnormal, excessive neuronal discharges detected by electroencephalographic recordings (EEG) in epileptic patients. EA is characterized by transient electrical events that deviate from normal background activity and reflect hypersynchronous neuronal firing, and is observed across multiple neurological conditions, including epilepsy and other disorders associated with cognitive impairment, such as autism. Using a seizure-free experimental model of chronic EA induced by a

single intrahippocampal injection of 0.74 mM kainic acid, we previously demonstrated persistent epileptiform discharges in hippocampal networks together with cognitive deficits in mice. To investigate the underlying circuit alterations, we characterized neuronal populations in CA1 and CA3 hippocampal regions, including pyramidal neurons and GABAergic interneurons, revealing region-specific alterations associated with EA.

Authors

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Reference number: T02-67

LONG-TERM BEHAVIORAL AND ENDOCANNABINOID ALTERATIONS FOLLOWING ADOLESCENT ALCOHOL AND THC CO-EXPOSURE

Adolescence is a critical period of brain development in which exposure to drugs of abuse may induce long-lasting neurobiological alterations. Among adolescents, binge drinking frequently co-occurs with cannabis use, although the consequences of this dual exposure on the endocannabinoid system and behavior remain poorly understood. The aim of the present study was to establish an experimental model of combined alcohol and Δ9-tetrahydrocannabinol (THC) exposure during adolescence and to investigate its long-term effects on behavior and the endocannabinoid system in adulthood.

Male C57BL/6J mice were divided into four experimental groups: control, alcohol, THC, and alcohol+THC. Animals underwent a voluntary binge-like alcohol consumption protocol combined with once-weekly THC administration (5 mg/kg, i.p.) from the second to the fourth week of the exposure period. Following a withdrawal period, behavioral analyses were performed to evaluate anxiety-like behavior and working memory using the Open Field, Light/Dark Box, Elevated Plus Maze, and Y-Maze tests. Animals were subsequently perfused, and brain tissue was collected for immunohistochemical analyses by light microscopy to evaluate cannabinoid receptor type 1 (CB1) and monoacylglycerol lipase (MAGL) expression in the prefrontal cortex, nucleus accumbens, CA1 hippocampal region, and basolateral amygdala.

Behavioral analyses revealed group-dependent trends in exploratory and anxiety-like behaviors, although no statistically significant differences were observed. Blood ethanol concentrations reached lower levels than expected under the present experimental conditions. Preliminary histological observations suggested region-specific differences in CB1 and MAGL immunolabeling patterns across experimental groups. Together, these findings suggest that adolescent alcohol and THC co-exposure may induce subtle behavioral and endocannabinoid alterations, highlighting the importance of exposure pattern and intensity in the long-term neurobiological consequences of dual substance use.

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Keywords: adolescence; binge drinking; alcohol; THC; dual consumption; endocannabinoid system; CB1; MAGL; hippocampus

Authors

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Reference number: T02-68

FROM DOPAMINERGIC DIFFERENTIATION TO SPINAL CORD REPAIR: CNT-BASED PLATFORMS FOR NEURAL RECONNECTION

The limited regenerative capacity of the central nervous system remains one of the major challenges in neuroscience and regenerative medicine. In this context, understanding and rebuilding neural circuits requires interfaces capable of modulating cell fate, organizing functional networks and restoring communication after injury. Here, we present an integrated translational strategy based on carbon nanotubes (CNTs) as conductive biointerfaces that, due to their electrical properties, nanoscale architecture and intimate interaction with neuronal membranes, can act across multiple scales of the central nervous system.

In human iPSC-derived neurons, CNT-based electroactive platforms promoted neuronal maturation and selectively enhanced dopaminergic differentiation, enabling real-time dopamine sensing from living neuronal networks. We then applied this concept into more physiologically relevant systems: conductive 3D honeycomb hydrogels integrating CNTs, where the synergy between microarchitecture and conductivity guided neuronal organization, neurite interconnection and the formation of functionally mature neural networks with enhanced calcium dynamics.

Finally, we translated these platforms to spinal cord injury, where conductive CNT-based implants acted as functional bridges across the lesion site and promoted locomotor recovery in vivo. Building on these results, we are currently advancing toward large-animal models to evaluate the translational potential of neural reconnection implants for spinal cord repair.

Overall, our work positions CNT-based neural interfaces as a versatile neurotechnology platform linking neuronal specification, functional network maturation and spinal cord reconnection within a unified translational framework.

Authors

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Reference number: T02-69

EXPLORATION OF HIPPOCAMPAL-CORTICAL CIRCUITS IN AN EPILEPTIFORM ACTIVITY MODEL USING HIGH-DENSITY MICROELECTRODE ARRAYS

Epileptiform activity (EA) consists of abnormal, excessive neuronal discharges detected by electroencephalography (EEG) and was first described in patients with epilepsy. Beyond epilepsy, EA also occurs in several neurological disorders, including Alzheimer's disease, autism spectrum disorder, attention-deficit/hyperactivity disorder, and traumatic brain injury. EA is strongly associated with cognitive impairment. Using an experimental model of EA based on a single intrahippocampal injection of kainic acid (KA), we observed the chronic persistence of hippocampal epileptiform discharges, the induction of aberrant neurogenesis, and impaired recognition memory in mice.

However, the mechanistic links between chronic EA and cognitive impairment remain unknown.
We hypothesize that EA induces functional alterations in neural network activity within the hippocampus and related structures, which may represent an underlying mechanism linking EA to cognitive impairment. To investigate this hypothesis, we used biosensing technologies capable of capturing large-scale neuronal interactions with high spatial and temporal resolution in acute mouse brain slices obtained 60-70 days after KA injection. High-density microelectrode arrays (HD-MEAs) overcome the limitations of conventional electrophysiology, providing a mesoscopic-scale view of interactions among neuronal assemblies. Here, we use HD-MEAs to characterize neuronal firing and network activity in hippocampal-cortical circuits in our EA model.
While spontaneous hippocampal network activity remains largely preserved, an EA phenotype emerges in cortical nodes, particularly in the entorhinal cortex, where firing and burst dynamics are increased. Moreover, when networks are challenged with the pro-convulsant drug 4-aminopyridine (4-AP), EA slices exhibit a more heterogeneous response, suggesting a functional disorganization of hippocampal-cortical circuits.

Authors
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Reference number: T02-70

THE ROLE OF THE LATERAL HABENULA IN MODULATING BIOLOGICAL RELEVANCE DURING AVERSIVE LEARNING

The ability of organisms to accurately assign valence and salience to environmental stimuli is a fundamental evolutionary process for survival. Within this framework, the Lateral Habenula (LHb) has emerged as a key neural substrate in the modulation of adaptive behavior, acting as a critical interface between forebrain structures and the monoaminergic systems of the brainstem. In the present study, we employed a bimodal experimental approach to investigate LHb function during the acquisition of aversive memories. Using the Fear Conditioning paradigm, we sought to characterize the LHb's involvement in the early stages of associative learning and in the long-term memory retrieval. This approach provides a comprehensive framework to explore whether the LHb acts as an active modulator of associative strength and to further our understanding of the circuit-level mechanisms that gate the consolidation of biologically relevant information in the brain.

Authors
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Reference number: T02-71

LOCUS COERULEUS A-SYNUCLEINOPATHY ALTERS NORADRENERGIC TRANSMISSION AND HIPPOCAMPAL ACTIVITY IN EARLY-STAGE PARKINSON'S DISEASE.

Although the clinical hallmark of Parkinson's disease (PD) is classically defined by motor impairment, non-motor symptoms such as cognitive and mood disturbances are now

recognised as a critical and early component of the disease. These features may precede motor manifestations by years and are strongly associated with dysfunction of the locus coeruleus (LC), the brain's main source of noradrenaline (NA). The LC is among the earliest regions to develop Lewy pathology and neurodegeneration, and its impairment can disrupt NA modulation of neuronal circuits, including dopaminergic networks. We set up a mouse model based on targeted overexpression of human α-synuclein (aSyn) in the LC by stereotaxic viral vector injection. Our aim was to characterise behavioural, functional, and structural alterations driven by early dysregulation of the LC–NA system, both locally and in projection areas such as the hippocampus (HC). Behavioural analyses revealed deficits in spatial learning and aversive memory, consistent with prodromal cognitive and emotional alterations in PD. At a structural level, aSyn mice showed aSyn spreading in LC projection regions, and phosphorylated aSyn accumulation in the LC, as well as disruption of the NA fibre network in the HC and increased GFAP-immunoreactive area in both LC and HC. In parallel, we evaluated hippocampal long-term potentiation (LTP) as a measure of synaptic plasticity and circuit function potentially affected by noradrenergic dysregulation. Overall, this work supports a key role for early LC dysfunction in non-motor PD alterations and support the noradrenergic circuit as a potential target for early intervention in the disease.

Authors

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Reference number: T02-72

AN INTEGRATED NEUROIMMUNE ASSEMBLOID MODEL TO ADVANCE NEURODEGENERATIVE DISEASE STUDIES

Brain organoids are three-dimensional cultures derived from human pluripotent or embryonic stem cells that recapitulate key genetic, biochemical, and molecular features of the human brain. They provide a powerful platform for studying human brain development and modeling genetic neurological disorders. However, their application to age-dependent neurodegenerative diseases remains limited, largely due to the absence of standardized methods for incorporating functional microglia, critical regulators of neuroinflammation and disease progression. Here, we describe a strategy for generating neuroimmune assembloids, brain organoids containing functional glial cells capable of mounting inflammatory responses. By introducing hematopoietic progenitor cells into developing brain organoids, we enable their in situ maturation into microglia-like cells that persist in culture for up to one month. These cells exhibit hallmark microglial behaviors, including morphological remodeling, migration, phagocytosis and transcriptional changes in response to inflammatory stimuli. Together, these immunocompetent-like brain organoids provide a promising and versatile platform for investigating neuroimmune interactions and neuroinflammatory mechanisms underlying
age-related neurodegenerative diseases.

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Reference number: T02-73

BRAIN-IMMUNE SYSTEM INTERACTIONS IN MAJOR DEPRESSION: ROLE OF B2 ADRENOCEPTORS IN MYELOID AND LYMPHOID CELLS.

Depression has been linked to sympathetic activity, inflammatory responses, and immune system imbalance, illustrating the bidirectional interconnection between mental health and immune function. These interactions can be readily observed in hematopoietic stem cells (HSCs), which are needed for immune homeostasis and are tightly regulated by sympathetic signaling. A recent study showed chronic stress alters bone marrow HSC function, promoting abnormal myeloid cell production, inflammation, and immune gene dysregulation [1]. It is well known that the β2-adrenergic receptor (ADRB2) is highly present in monocytes and T lymphocytes. Its activation by adrenaline and noradrenaline typically results in immunosuppression, including reduced cytokine expression via the cAMP-PKA pathway and other non-traditional pathways [2]. In addition, postmortem studies reported increased β-adrenoceptor expression in the prefrontal cortex (PFC) of suicide victims with major depression (MD) [3]. Moreover, chronic antidepressant treatment has been shown to reduce β-adrenergic receptor expression in the rat brain [4]. Together, these observations suggest that altered adrenergic signalling may play a key role in MD pathogenesis. Therefore, the present work hypothesizes that increased ADRB2 expression in immune cells may represent a compensatory mechanism that counters inflammatory processes, leading to antidepressant-like effects in MD.

This project aims to investigate β2-adrenergic receptor expression in immune cells to better characterize neuroimmune interactions in major depressive disorder (MDD), assessing its association with inflammation and treatment response. In addition, we aim to study potential alterations linked to illness or clinical response upstream in undifferentiated hematopoietic progenitors, while also obtaining preliminary insights into adrenergic-receptor expression in mouse stress models.

Moderate and severe MD patients diagnosed according to the Montgomery–Åsberg Depression Rating Scale (MADRS >20) were recruited by psychiatrists from several hospitals. Blood samples were collected at baseline (T1) and follow-up after treatment (antidepressants, esketamine, or electroconvulsive therapy) (T2). From these samples, monocyte subsets (CD14+; CD16-/CD14+; CD16+) and T-lymphocytes (CD3+) were isolated by flow cytometry sorting from peripheral blood mononuclear cells (PBMCs). Gene expression analyses (ADRB2, ADRA2B, and IL6) were performed for each cell sorted type by real-time quantitative PCR (RT-qPCR). Statistical analyses were performed using two-way ANOVA, followed by appropriate post hoc multiple-comparison tests. In parallel, RNA sequencing (RNA-seq) was performed on hematopoietic progenitors (CD34+) from a small group of MD patients and healthy patients (HC). The study was conducted in accordance with approved ethical protocol. Additionally, secondary analysis of publicly available scRNA-seq data from chronic unpredictable mild stress (CUMS)-exposed mice revealed reduced beta-adrenergic signaling in myeloid cells, suggesting a diminished activation state compared to control animals.

MD patients revealed a significant increase in ADRB2 mRNA expression in classic monocytes compared with HC ($p < 0.001$), with high levels in both moderate and severe patients. In parallel, increased IL6 mRNA expression was observed in classical monocytes ($p < 0.01$), particularly in patients with moderate depression. Longitudinal

analyses showed that IL6 mRNA levels decreased in responder patients at follow-up compared to baseline (p = 0.025).
These findings support that ADRB2 upregulation may constitute a compensatory mechanism to counteract inflammation and promote resilience to chronic stress, highlighting this pathway as a potential therapeutic target for immune-mediated depressive disorders.
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Authors
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Reference number: T02-74

BONE MARROW MYELOPOIESIS DYSFUNCTION IN ALZHEIMER'S DISEASE

Accumulating evidence suggests that both innate and adaptive immunity play crucial roles in combating Alzheimer's disease (AD). Specifically, enhancing the homing of monocyte-derived macrophages to the affected brain has been shown to reduce local inflammation, decrease proteinopathy, rescue neurons, and slow-down cognitive decline. However, the factors limiting their spontaneous recruitment remain unclear. Using multi-omics techniques, we identified impaired myelopoiesis and monocyte development in AD patients and mouse models. While not the primary cause of the disease, this impairment is associated with disease progression. In the 5xFAD mouse model, monocyte differentiation was found to be disrupted due to a maladaptive bone marrow (BM) response, driven by type I interferon (IFN-I) signaling. A similar phenotype was found in circulating monocytes from AD patients compared to healthy controls. Blocking IFN-I with monoclonal antibodies or using chimeric 5xFAD mice reconstituted with BM from mice lacking the IFN-I receptor (IFNAR1) alleviated myelopoiesis dysfunction, normalized monocyte phenotypes, and reduced disease manifestation. The improvements in myeloid function were accompanied by an increased homing of monocyte-derived macrophages in the 5xFAD brain. Our results reveal an unexpected dysfunction in BM myelopoiesis in neurodegeneration and reinforce the emerging concept that neurodegenerative diseases are not confined to the brain but also encompass the immune system.

Authors

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Reference number: T02-75

NEUROPROTECTION IN AN ANIMAL MODEL OF PARKINSON'S DISEASE THROUGH SYSTEMIC TARGETING OF INHIBITORY IMMUNE CHECKPOINT PATHWAYS

Local brain inflammation is currently considered an important key contributor to Parkinson's disease (PD) progression, similar to other neurodegenerative diseases, alongside alpha-synuclein (α-syn) aggregation. It is not yet clear what drives this inflammatory process and how it could be arrested. Emerging data from other neurodegenerative conditions suggest that these diseases are not confined to the brain, but also involve dysfunction of the immune system. Harnessing the peripheral immune system was found to be beneficial in arresting disease conditions in multiple models. Here, we hypothesized that this approach might be applicable to PD. We used a model of long-term α-syn overexpression in the nigrostriatal pathway of mice, using an adeno-associated virus as delivery vector (AAV-Syn), which induced dopaminergic neuron loss and motor deficits. We found that transient depletion of systemic Tregs during the active phase of neurodegeneration reduced severity of disease manifestation following α-syn overexpression. These results encouraged us to adopt the therapeutic approach of transiently blocking the inhibitory PD-1/PD-L1 immune checkpoint pathway, previously found to be effective in animal models of dementia, including amyloidosis and tauopathy, through a mechanism involving recruitment of monocyte-derived macrophages and Tregs to the brain. We found that a single systemic treatment with anti-PD-L1 antibody, administered 2 or 3 weeks after AAV-Syn injection, led to improved motor function, as assessed 5 or 6 weeks later using the pole and bar tests. In addition, we observed a robust protective effect on the survival of dopaminergic neurons. Overall, this therapeutic approach appears to be clinically translatable, suggesting that PD may be amenable to therapies targeting immune checkpoints.

Authors

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Reference number: T02-76

T CELL – MICROGLIA COMMUNICATION FOR NEUROPROTECTION IN A MOUSE MODEL OF PARKINSON'S DISEASE

Neuroinflammation is a major contributor to the progression of Parkinson's disease (PD). Degeneration of dopaminergic neurons disrupts glial homeostasis and promotes T cell infiltration into the midbrain, triggering an inflammatory reaction that may further exacerbate cell loss. However, it remains unclear whether the immune system and inflammation cause neuronal death, how glial and immune cells contribute to either neuronal protection or damage and at which stages of the neurodegenerative process they play their primary role. Here, we hypothesize that microglia and T cells establish a negative feedback loop that can be modulated to induce neuroprotection. Using the MPTP mouse model of PD, we observed reduced infiltration of CD4+ and CD8+ T cells in the midbrain, while regulatory T cells (Treg) were specifically elevated. Unexpectedly, selective depletion of Treg, using Foxp3DTR mice, decreased dopaminergic degeneration. This neuroprotective effect was accompanied by an increase in myeloid,

CD4+ and CD8+ T cells, which subsequently acquired an exhausted phenotype within the midbrain. Depletion of Treg enhanced microglia phagocytic activity while reducing astrocytic phagocytosis. Importantly, IL-2 blockade abolished both the neuroprotective effect mediated by Treg depletion and the associated glial phagocytic response. Under these conditions, glial phagocytosis shifted towards increased microglia-neuron interactions with increased astrocytic phagocytic activity. In addition, selective depletion of CD8+ T cells using a monoclonal antibody prevented the neuroprotective effect induced by Treg depletion. Overall, our data uncover a previously unrecognized immune-glial axis regulating dopaminergic degeneration in PD. Modulation of Treg-mediated immune suppression reshapes microglial phagocytic activity and CD8+ T cell function, ultimately promoting neuroprotection.

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Reference number: T02-77

IMAGING THE CHANGES IN BLOOD-BRAIN BARRIER PERMEABILITY AFTER PRECLINICAL ISCHEMIC STROKE

Ischemic stroke induces matrix metalloproteinase (MMP) activation, leading to blood–brain barrier (BBB) disruption and neuronal damage. However, the mechanisms and temporal evolution of BBB disruption during subacute phase of ischemic stroke remain poorly understood. This study evaluates BBB integrity, neuroinflammatory response, and MMP activity following experimental stroke.

Male C57BL6/J mice (N=75) were subjected transient middle cerebral artery occlusion (tMCAO; 60 min). BBB permeability was assessed ex vivo using Evans Blue (EB) extravasation at baseline and at 1, 3 and 6 hours, and 1, 3 and 7 days post-tMCAO. In vivo BBB disruption was evaluated by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) using gadolinium. Neuroinflammation was analyzed ex vivo by immunohistochemical detection of vascular cell adhesion molecule-1 (VCAM-1). Finally, MMP activity was quantified by gelatin zymography.

Ischemic mice displayed a biphasic pattern of BBB disruption, characterized by an acute opening at 1-hour post-tMCAO, followed by partial recovery and a secondary disruption at day 7, as indicated by increased Ktrans values (DCE-MRI) and EB extravasation at these time points. VCAM-1 expression was markedly upregulated in ischemic cerebral vessels, indicating sustained vascular inflammation during subacute stroke.

In parallel, neuroinflammation is currently being investigated using the TSPO radiotracer [^{18}F]-DPA-714. In contrast zymography showed a peak increase in MMP-9 activity at 24 hours after stroke, followed by a progressive decline during the subsequent weeks. These findings demonstrate a biphasic BBB opening after cerebral ischemia, with early disruption mediated by MMP activation and delayed breakdown likely driven by inflammatory processes during the subacute phase.

Authors

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Reference number: T02-78

MRNA ANALYSIS UNCOVERS ALTERED FUNCTION OF PLASMACYTOID DENDRITIC CELLS IN MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system in which plasmacytoid dendritic cells (pDCs) may contribute to disease pathogenesis. We analyzed peripheral pDCs from untreated relapsing-remitting MS patients and healthy controls using bulk RNA sequencing, followed by functional enrichment, single-cell validation, and genetic regulatory analyses. We identified a broad transcriptional signature in MS pDCs enriched in antigen presentation, MHC class II pathways, and T cell activation. Single-cell analysis confirmed altered pDC subpopulation distribution in patients, with one cluster showing increased activation of CD4+ T cell–related pathways. A multivariable model based on selected genes showed strong ability to distinguish MS patients from healthy controls. Cis-eQTL analysis revealed multiple regulatory variants affecting disease-associated genes, with the MHC class II region showing the strongest genetic regulatory signal. This included key genes involved in antigen presentation and the presence of the high-risk HLA-DR15 haplotype, reinforcing the importance of this locus in MS susceptibility. Overall, our results highlight significant transcriptomic and genetic alterations in pDCs in MS, supporting a role for dysregulated antigen presentation in disease mechanisms and suggesting potential biomarker relevance.

Authors

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Reference number: T02-79

EVALUATION OF POTENTIAL ANTI-INFLAMMATORY EFFECTS OF PSILOCYBIN IN A PHARMACOLOGYCAL MOUSE-MODEL OF ACUTE INFLAMMATION

Major depressive disorder (MDD) is one of the most prevalent mental illnesses worldwide. However, biological mechanisms underlying its development remain unclear. Elevated pro-inflammatory cytokine concentrations have been observed in peripheral blood samples of MDD patients, suggesting a link between inflammation and the pathophysiology of depression. Long- and new fast-acting antidepressants, such as psilocybin, have been suggested to produce antidepressant effects trough anti-inflammatory properties. In this context, this study aimed to evaluate the anti-inflammatory effects of psilocybin measuring both peripheral and central cytokine levels in a mouse model of acute inflammation.

Adult C57BL/6J male and female mice were randomly administered with Polyinosinic:polycytidylic acid [Poly(I:C)] (2.5 mg/kg, i.p.)—a synthetic analogue of double-stranded RNA that mimics viral infection and activates the innate immune response— or saline. Three hours after Poly(I:C) administration, psilocybin (5 mg/kg, i.p.) or saline were administered. Animals were sacrificed and tissue was harvested six hours after the first injection. Milliplex immunoassay was performed to evaluate peripheral cytokine (IFN-γ, IL-1α, IL-1β, IL-2, IL-6, IL-10, and TNF-α) concentration in blood serum. Central cytokine mRNA levels (for Ifng, Il1b, Il6 and Tnf) were evaluated using TaqMan® Gene Expression Assays for real time polymerase chain reaction (qPCR) in brain cortices.

In blood-serum, administration of Poly(I:C) led to a significant elevation in the levels of IFN-γ, IL-6, IL-10, and TNF-α ($F(1,45)=55.78$, $p<0.0001$; $F(1,42)=83.44$, $p<0.0001$; $F(1,43)=65.55$, $p<0.0001$; $F(1,46)=79.06$, $p<0.0001$, respectively). Psilocybin treatment did not significantly modify these inflammatory markers, except for IFN-γ, where it reversed the Poly(I:C)-induced elevation in both male and female mice ($F(1,45)=5.753$, $p=0,0207$). In brain cortex, Poly (I:C) significantly enhanced Il6, Il1b and Tnf mRNA expression ($F(1,39)=22.41$, $p<0.0001$; $F(1,36)=16.43$, $p=0.0003$; $F(1,42)=129.9$, $p<0.000$, respectively) when compared to control, but did not affect Ifng. However, psilocybin administration did not modulate mRNA expression for any of the selected genes.
These findings suggest a peripheral immunomodulatory effect of psilocybin and highlight the need for future studies using chronic inflammation models to further characterize its anti-inflammatory potential.
This work was supported by MCIN/AEI/10.13039/501100011033 (PID2021- 123508OB-I00) and the Department of Education (IT-1512–22) of the Basque Government. N.M-A received a predoctoral fellowship from the Basque Government (PRE_2022_1_0256).

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Reference number: T02-80

ICAM1 AS A CONTRIBUTOR TO HUMAN ASTROCYTE-DRIVEN NEURON VULNERABILITY IN AD

Astrocytes are central players in the pathogenesis of Alzheimer's disease (AD), undergoing profound changes in gene expression, morphology, and function. These changes can significantly alter the astrocyte-neuron microenvironment, yet remain poorly
understood in human astrocytes. Using human pluripotent stem cell (hPSC)-derived astrocytes, we established an in vitro approach to investigate astrocyte responses to AD-relevant challenges. Our data reveal that conditioned medium from astrocytes (ACM) exposed to AD associated factors induces significant neuronal death, in contrast to the lack of toxicity observed when hPSC-derived neurons are directly exposed to the same challenges. This highlights a secreted, astrocyte-dependent mechanism of neurotoxicity. Multiomic analyses of astrocytes treated with these AD-relevant factors revealed significant dysregulation of pathways involved in inflammatory processes, extracellular matrix remodeling, and neuronal maintenance. Among the secreted factors, ICAM1 was identified as a key candidate mediating astrocyte-induced neurotoxicity. These findings provide new insights into the role of human astrocytes shaping a neurotoxic environment
in AD and identify ICAM1 as a potential driver of astrocyte-neuron interactions in neurodegeneration. In addition, this model serves as a valuable platform for further investigation into astrocyte-mediated mechanisms of AD pathology.

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Reference number: T02-81

EFFECT OF THE METALLOPORTEINASE INHIBITOR ACT-03 ON THE ALTERATIONS INDUCED BY TRAUMATIC BRAIN INJURY ON THE NEUROGENIC NICHE.

Traumatic brain injury (TBI) is one of the leading causes of long-term neurological disability, often resulting in persistent cognitive and behavioral impairments. Among the different brain regions affected, the hippocampus is especially vulnerable to secondary injury processes after trauma. Neuroinflammation plays a major role in this vulnerability, contributing to neuronal dysfunction, cell death, and the development of long-term pathological outcomes.
Matrix metalloproteinases (MMPs) participate in neuroinflammatory cascades and blood–brain barrier disruption after TBI, making them promising targets for therapeutic intervention. Here, we focus on the potential use of the synthetic MMP inhibitor ACT-03 to preserve hippocampal integrity following TBI.
In this study, we used the Controlled Cortical Impact (CCI) model in rodents to reproduce traumatic brain injury. The effects of ACT-03 treatment were assessed at different post-injury time points to evaluate both acute and subacute responses.
To evaluate treatment efficacy, several cellular and molecular markers related to neuroinflammation and neurodegeneration were analyzed. Microgliosis and astrogliosis were assessed through immunohistochemical analysis of specific markers, while apoptosis levels were evaluated to determine the extent of neuronal damage and cell death. In addition, the expression of inflammatory and neurodegenerative mediators was analyzed to further characterize the therapeutic effects of ACT-03.

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Reference number: T02-82

EBV INFECTION DYSREGULATES THE EXPRESSION AND METHYLATION PROFILE OF MITOCHONDRIA-RELATED MULTIPLE SCLEROSIS RISK GENES IN B CELLS

Background
Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus closely associated with multiple sclerosis (MS). To meet its own replication needs, EBV induces an increased energy demand in B cells that is carried out by the mitochondrion, a cellular organelle responsible for the energy production in cells. An over-activation of the mitochondria, however, could lead to its dysfunction and ultimately, to symptoms of fatigue.

Objectives
To determine the transcriptome and methylome alterations induced by EBV infection in the mitochondrial landscape by analyzing existing RNA-seq and bisulfite-seq databases obtained from resting B cells and EBV-infected B cells.
Methods
The transcriptome of mitochondrial genes were studied by RNA-seq in paired CD19+ B cells and EBV-infected B cells [i] during the first 14 days post-infection and [ii] in

lymphoblastoid cell lines (LCLs). The DNA methylation profile of resting B cells and LCLs was analyzed by whole genome bisulfite-seq. Differentially methylated and/or expressed genes were analyzed and later filtered for the 37 mitochondrial DNA-encoded genes and the 1590 mitochondria-related nuclear DNA-encoded genes.
Results
Mitochondrial DNA displayed lower methylation levels in 11 CpGs located within the D-loop region, ND1 and COX1 genes in resting B cells vs LCLs (Padj <0.05). Transcriptome analysis revealed dysregulation of the mitochondria-related MS risk genes PRDX5, CMC1, CYP27B1 and VDR during the course of EBV infection (Padj <0.05), while the promoter of the MS risk gene PRR5L showed hypomethylation in LCLs vs resting B cells (P=0.049).
Conclusions
EBV infection induces profound changes in the methylome and transcriptome of the mitochondrial landscape in B cells, including in mitochondria-related MS risk genes, which might be contributing to the pathophysiology of the disease, including symptoms of fatigue.

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Reference number: T02-83

TARGETING NEUROINFLAMMATION TO PRESERVE NEURONAL NETWORK ACTIVITY IN EPILEPSY

Epilepsy is the third most common chronic brain disorder and is described as a chronic brain disorder where normal neurodynamics transform into pathodynamics after a process of epileptogenesis, triggering recurrent spontaneous unprovoked electrical seizures. These seizures occur in a positive feedback loop of various factors, including neuroinflammation. Among other pathways involved in neuroinflammation, evidence shows the involvement of the JAK/STAT signaling pathway in epilepsy, although knowledge of this participation is limited.
This project is based on evidence suggesting the possible relevance of the STAT3 transcription factor in epilepsy. In this project, an in vivo model of medial temporal lobe epilepsy (MTLE) was tested where WP1066 (a STAT3 inhibitor) was administered, with the hypothesis that this inhibitor could attenuate the alterations observed in the hippocampal neurogenic niche of the MTLE murine model. To this end, MTLE was induced in mice by injecting kainate, with WP1066 treatment beginning the day before the injection, and continuing for up to 14 days. The observed alterations were analyzed by immunofluorescence.
The murine model of MTLE due to kainate mimics the alterations in the hippocampal neurogenic niche observed in MTLE patients. STAT3 blockade efficiently reduced the in vivo granular layer dispersion; prevented the increase in neuroinflammatory markers and avoided the degeneration of GABAergic neurons; and reversed the neurogenic capacity as well as the dispersion of immature neurons in the granular layer. This project provides evidence that blockade of the STAT3 signaling pathway by WP1066 may prevent some of the hippocampal neurogenic niche alterations characteristic in MTLE. The development of compounds that block STAT3 could result in therapies to modify the characteristics of epilepsy and not only treat the symptoms.

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Reference number: T02-84

MULTIPLE SCLEROSIS RESHAPES TRANSCRIPTIONAL AGING TRAJECTORIES

Aging is widely known to reshape transcriptional programs associated with pathways such as immune regulation, inflammation, and cellular homeostasis. Similarly, multiple sclerosis (MS) is characterized by distinct disease-associated gene expression signatures. However, despite increasing evidence linking biological aging to MS progression, how aging modifies transcriptional trajectories in people with MS (pwMS) remains poorly understood.

To address this, we analyzed gene and miRNA expression profiles from 150 individuals, including 75 healthy controls (HC) and 75 pwMS. First, we assessed feature-wise associations with age independently in HC and pwMS using Spearman correlation, enabling the characterization of cohort-specific aging patterns. Second, we applied a penalized regression approach (LASSO) to identify features with predictive value for age in each group, revealing shared and group-specific molecular signatures of aging. Third, we performed differential expression analysis using DESeq2 to evaluate both the main effect of disease status and the interaction between age and condition, allowing the identification of features whose expression trajectories differ with aging between cohorts.

Overall, our results indicate that MS is associated with altered transcriptional aging dynamics, affecting both age-related expression patterns and molecular signatures predictive of aging. Together, these findings support the existence of distinct transcriptional aging trajectories in pwMS, characterized by altered age-associated expression patterns and disease-specific regulatory signatures. Additionally, we identified candidate biomarkers linked to disease-associated aging processes, including a microRNA whose expression declines with age exclusively in pwMS and which has been previously associated with remyelination mechanisms.

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Reference number: T02-85

METHODS FOR ASSESSMENT OF MOTIVATIONAL DISORDERS IN MULTIPLE SCLEROSIS

Multiple Sclerosis (MS) causes a wide variety of symptoms, from evident motor disabilities to subtle mental health diseases, being episodes of anxiety, depression and social phobia frequent in MS patients. In the brain, the prefrontal cortex – nucleus accumbens pathway (PFC-NAc), included in the dopaminergic mesolimbic system, plays a critical role in reward, anhedonia and motivation disorders and it has been reported that physical activity has a positive impact in the reward pathway of subjects accepting exercise voluntarily. Consistently, frequent training is recommended to MS patients to retain and restore as much as possible their musculoskeletal system. To explore potential links and correlations between motor symptoms, motivation, cognitive

impairments and physical exercise we have taken advantage of automated behaviour phenotyping systems that enable the quantification of displacement, access and use of a rotating wheel (TSE Systems Phenomaster) and performance of cognitive tasks and associative memory, progressive learning and food preference (TSE System IntelliCage). Mice were housed in IntelliCage along the duration of the experiment in a social context and routinely were individually transferred to Phenomaster for voluntary physical training sessions. The analysis of a pilot control mice group has shown a fast adaptation to these animal monitoring cages with a strong interest on the rotating wheel and learning of behavioural challenges. In addition to this, at the end of the experiment, the neuronal firing properties of Medium Spiny Neurons was evaluated by means of calcium imaging reported by Fluo-4 loaded in NAc slices under a fluorescence microscope. Altogether, these methods are aimed to provide the baseline of the functioning of the neurobiological substrate and cognitive performance of mice that might be disrupted in an Experimental Autoimmune Encephalomyelitis mouse model, the primary preclinical tool used to study MS.

Authors

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Reference number: T02-86

IMPROVING GENETIC DIAGNOSIS OF INHERITED RETINAL DYSTROPHIES: FROM SYSTEMATIC REANALYSIS TO FUNCTIONAL ASSAYS

Inherited retinal dystrophies (IRD) are a heterogeneous group of diseases characterized by progressive visual impairment, with over 300 genes implicated in their pathogenesis. Consequently, despite advances in Next-Generation Sequencing (NGS), a significant portion of patients with IRD remain without a molecular diagnosis. The continuous evolution of genomic databases and the discovery of novel disease mechanisms require periodic re-evaluation of unsolved cases.

To advance the molecular genetic characterization, we performed a multidimensional reanalysis of patients in our cohort previously classified as inconclusive. Our strategy followed three main axes: 1) Data re-evaluation, integrating updated population frequencies and pathogenicity scores; 2) Study of new candidate genes, focusing on genes with high expression levels in the human retina; and 3) Study of regulatory regions, specifically targeting variants in 5'UTRs. Furthermore, candidate variants—specifically splicing and missense variants of uncertain significance—are currently being characterized via midigene systems and cell-based functional assays using full-length cDNA, to determine their pathogenic impact.

The database updates led to the identification of the causative variants in 4 additional families of the cohort. The integration of retinal expression data and regulatory region analysis contributed to the identification of one potential new candidate gene. Furthermore, in vitro functional studies are currently in progress to validate candidate variants. We anticipate that these results will further increase the overall diagnostic yield and provide definitive molecular confirmation for additional inconclusive cases.

Our study reinforces the necessity of recurrent genomic re-evaluation and the integration of functional evidence to increase the diagnostic yield in IRD cohorts.

Authors
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Reference number: T02-87

PERIPHERAL AUDITORY SYSTEM DEGENERATION AS A HALLMARK OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) has been increasingly associated with auditory dysfunction, suggesting that sensory deficits may contribute to cognitive decline beyond age-related hearing impairment alone. While central auditory alterations have been extensively investigated, the impact of AD-related neurodegeneration on peripheral auditory structures remains poorly understood. In this study, we evaluated cochlear histopathology in 24-month-old 3×Tg-AD mice to investigate structural alterations associated with auditory dysfunction in AD.
Histological analyses revealed near-complete loss of spiral ganglion neurons (SGNs) in aged 3×Tg-AD mice compared to wild-type controls, accompanied by a pronounced reduction in cochlear afferent nerve fibres. Nissl staining further revealed severe morphological deterioration and neurodegenerative features in surviving neurons from AD mice. Immunofluorescence analyses further supported neuronal alterations within the auditory ganglia. Moreover, Sirius Red staining ahowed increased fibrotic deposition throughout cochlear tissues in 3×Tg-AD mice, suggesting extensive tissue remodeling associated with neurodegeneration. Consistently, TUNEL assays showed elevated apoptotic activity, particularly in regions surrounding the spiral ganglion, indicating ongoing cellular degeneration.
These findings demonstrate a strong association between peripheral neurosensory loss and AD, including neuronal loss, apoptosis, and fibrotic remodeling within the cochlea. Our results support the existence of a pathological link between AD and peripheral auditory system impairment and highlight cochlear alterations as potential early indicators of neurodegenerative disease progression.

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Reference number: T02-88

EVALUATING THE THERAPEUTIC EFFICACY OF MP-004 IN A MOUSE MODEL OF STARGARDT'S DISEASE.

Topical administration of MP-004 effectively mitigates functional retinal impairment in a severe STGD model. Dual modulation of OS and Ca2+ represents a promising, non-invasive therapeutic strategy for STGD and other inherited retinal dystrophies. The observed sexual dimorphism highlights the importance of gender-specific considerations in preclinical IRD research.
Our research group is developing a non-invasive therapeutic strategy for inherited retinal dystrophies (IRDs) using ocular instillation of MP-004, a small molecule that

modulates oxidative stress (OS) and maintains calcium (Ca^{2+}) homeostasis—two key pathogenic mechanisms shared by multiple retinal disorders, including Stargardt disease (STGD). STGD, the most common hereditary macular dystrophy, is caused by biallelic mutations in ABCA4, leading to impaired transport of vitamin A derivatives, lipofuscin accumulation, increased OS, and Ca^{2+} dysregulation, ultimately resulting in photoreceptor death.
This study aimed to assess MP-004 efficacy in Abca4-/- Rdh8-/- mice subjected to acute light-induced phototoxicity to accelerate retinal degeneration. Animals received topical MP-004 three times per week, and retinal function was monitored longitudinally by full-field electroretinography (ERG) to evaluate scotopic and photopic responses. A sex-related difference was observed: female mice exhibited lower baseline ERG amplitudes and greater sensitivity to phototoxicity compared to males. Notably, MP-004 treatment significantly mitigated functional decline, as treated mice preserved ERG responses relative to vehicle controls, indicating reduced photoreceptor loss.
These findings support MP-004 as a promising candidate for IRDs, including STGD, by targeting shared pathogenic pathways and providing functional protection through topical delivery.

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Reference number: T02-89

EXPRESSION OF HABENULAR CB1 RECEPTORS AND NEUROGLIAL ADAPTATIONS IN A MOUSE MODEL OF PRIMARY DEMYELINATION: IMPLICATIONS FOR PSYCHIATRY COMORBIDITIES ASSOCIATED TO MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic inflammatory disease of the brain and spinal cord characterized by demyelination and neurodegeneration affecting both white and grey matter structures. Depression and anxiety are highly prevalent inMS and significantly influence patient´s quality of life. The habenular complex (HbCpx) is a highly conserved brain structure composed by medial (MHb) and lateral (LHb) habenular subnuclei that critically regulate emotional and motivational states. Habenular hyperactivity has been consistently associated to depression and deficient emotional control through mechanisms that involve increased bursting activity of MHb and LHb neuronsand aberrant astrocyte-neuron interactions. Cannabinoid CB1 receptors (CB1R) fine-tune synaptic inputs and astrocytic functionsin the HbCpx, with emerging implications in depression and anxiety disorders. Nevertheless, the functional roles of habenular CB1R in the pathophysiology of psychiatric comorbidities associated to MS remain unknown. Here, we investigated the expression of habenular CB1R in the cuprizone mouse model of primary demyelination. Cuprizone feeding induced anxiety-like behaviors in the open field test. Immunofluorescence experiments targeting APC, GFAP, S100β, Iba1 and showed oligodendrocyte loss, increased astrocyte numbers/reactivity and microglia activation affecting the LHb and/or MHb in CPZ-treated mice. CB1R immunolabeling revealed by DAB immunohistochemistry suggested downregulation of the receptor proteinin the LHb. These results demonstrate that the HbCpx is sensitive to toxin-

induced demyelination and points to specific roles for habenular CB1R in the pathophysiology of psychiatric comorbidities associated to MS.
Keywords: habenular complex, CB1receptors, multiple sclerosis, cuprizone.
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6.3 TOPIC 03: NOVEL TOOLS AND METHODS

Reference number: T03-1

IN VIVO TWO-PHOTON IMAGING OF REPERFUSION INJURY IN A MOUSE MODEL OF ISCHEMIC STROKE

Reperfusion injury is a major determinant of tissue damage and functional outcome in ischemic stroke, yet its underlying mechanisms remain poorly understood mainly because limitations of experimental models. To address this gap, we investigated reperfusion injury using novel stroke models combined with in vivo two-photon imaging. Stroke models were induced via ligation of the middle cerebral artery branch that irrigate the somatosensory cortex. Two approaches were developed: permanent stroke model (pStroke), where the perfusion is not restored, and transient stroke model (tStroke), where the perfusion is restored after 60 minutes.
Reperfusion preserved brain tissue after acute ischemic injury as shown by cresyl violet and Fluoro-Jade C staining. Moreover, neuronal activity, although aberrant, exhibited a lower degree of alteration compared with neurons under complete anoxia as observed by two-photon imaging. However, we observed that the vascular response was absent or delayed following reperfusion. As expected, blood leakage was greater in transient occlusions and persisted longer in the injured area as demonstrated by Evans blue assay and two-photon imaging. Nevertheless, reperfusion resulted in the extravasation of smaller blood molecules suggesting partial blood–brain barrier (BBB) conservation at the neurovascular unit level. Indeed, we observed that BBB component were more preserved when reperfusion happens.
Our results suggest that, despite partially limiting neuronal damage, blood reperfusion induces long-term alterations in the blood–brain barrier (BBB) integrity. This BBB disruption may explain the impaired vascular response, persistent blood leakage and worse behavioral outcomes observed following reperfusion. Thus, our findings highlight the relevance of reperfusion injury in ischemic pathology.

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Reference number: T03-2

MAPPING THE STRUCTURE AND FUNCTION OF WHOLE-MOUNT RETINAL ORGANOIDS

Purpose. Cell therapy is one potential therapeutic avenue for late-stage retinal degenerative diseases, such as Retinitis Pigmentosa and Age-related Macular Degeneration disease. Photoreceptor transplantation faces significant challenges in obtaining high-quality and mature photoreceptors in vitro that can effectively integrate and function, facilitating regeneration. For designing cell-based therapies we will produce retinal organoids (RO) from human induced pluripotent stem cells (ihPSC). To enhance our understanding of RO maturation in a 3D and dynamic context, we aim to characterize the structure and function of retinal neurons. This will help identifying the optimal timing for harvesting photoreceptors for subsequent implantation. In this work, we focus on the characterization of RO derived from ihPSC from healthy and vision-impaired individuals, specifically Retinitis
Pigmentosa type 25.

Methods. To study the neuronal network conforming the retina, we tracked calcium activity in 3D with light-sheet fluorescence microscopy (LSFM). In LSFM, the sample is irradiated with a thin sheet of light and the fluorescence generated is captured with a camera. This optical configuration allows an in vitro, fast tracking of dynamics (10 Z-planes at 5 Hz; 200 ms/volume) occurring on a volumetric scale (600x830x100 µm) with minor or none photo-toxic or photo-damage effects. For visualizing the changes in the intracellular calcium influxes, we infected the RO with viral vectors encoding for the calcium indicator GCaMP6s. The changes in fluorescence intensity were quantified and analysed in a 3D fashion to determine the pattern and frequency of calcium signals in the neuronal network.
Subsequently to the life dynamic recordings, and to characterize the structure of whole-mount mature ROs (up to day 250) in their natural 3D architecture, we optimized an optical clearing method (FluoClear BABB) in combination with improved antibody permeabilization.
Results. We imaged in high-resolution the calcium activity of RO derived from healthy and vision impaired donors and quantified differences in the functional communities of neurons by using transfer entropy. Using our clearing protocol, we were able to identify the distinct retinal cell types and their spatiotemporal interaction within the organoid using specific antibodies. We described the morphology and population of the three neuron-path that provide the direct route for visual information transmission: cone and rod photoreceptors, bipolar and ganglion cells.
Conclusions. Retinal organoids are highly promising as an in vitro model for studying retinal diseases. Our findings shed more light on the temporal, spatial, and functional organization of retinal cells within the organoid. Our LSFM can be applied to study fast events (milliseconds) occurring in millimetre range samples with cellular resolution.

Authors

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Reference number: T03-3

EXPERIMENTAL AND ANALYTICAL MODEL FOR TWO PHOTON CALCIUM IMAGING IN HEALTHY AND DISEASED RETINAL CIRCUITS

Retinal degenerative diseases are characterized by the progressive loss of photoreceptors and represent a major cause of irreversible vision loss. Among them, age-related macular degeneration (AMD) is one of the leading causes of blindness in the aging population. Although structural alterations during degeneration have been extensively described, the functional impact on retinal network activity remains poorly understood. One of the main limitations is the lack of robust experimental approaches to monitor neuronal activity in degenerating retinal tissue.
In this work, we establish a functional imaging pipeline based on two-photon calcium imaging to study retinal circuit activity with cellular resolution. We use Thy1-GCaMP6s transgenic mice to record activity in the retinal ganglion cell layer. This framework is designed to investigate functional alterations in a chemically induced degeneration model that reproduces key features of AMD.
Retinal explants are prepared under dim red-light conditions and maintained in oxygenated Ames' medium. Imaging is performed with a bespoke two-photon excitation

fluorescence microscope, enabling stable long-duration recordings. Visual stimulation is provided by a 380 nm LED, including chirp stimuli, while calcium signals are acquired at 5 frames per second, over a 300 × 300 μm² field of view. This approach reliably detects light-evoked responses across ganglion cells. Automated analysis, including segmentation, signal extraction, and clustering, reveals distinct functional groups. This platform provides a basis to quantify how degeneration alters retinal activity and organization, offering insight into mechanisms of visual dysfunction.

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Reference number: T03-4

HYDROGEL-BASED BIOSENSORS FOR THE DIAGNOSIS OF NEURODEGENERATIVE DISEASES

Early diagnosis of neurodegenerative diseases is essential for patient survival and successful prognosis [1]. Research efforts are focused on the non-invasive detection of neurological disease biomarkers, as several promising analytes, including p-tau, Na+ and salivary lactoferrin, have been identified in accessible biofluids such as saliva, sweat and interstitial fluid, offering a viable alternative to cerebrospinal fluid sampling [2-4]. However, most commercial bioelectronic devices rely on rigid, dry, and synthetic materials, which differ significantly from the nature of living tissues and hinder the development of flexible and implantable biosensors [5,6]. Multifunctional hydrogels address these challenges; their viscoelastic, porous, and water-rich 3D networks mimic the extracellular matrix, enabling the stable encapsulation of bioreceptors and enhancing sensitivity [6].

This work aims to develop a biocompatible hydrogel with optimized mechanical, optical, and electrochemical properties. A poly (ethylene glycol) diacrylate (PEGDA) and acrylic acid (AA) hydrogel incorporating an active bioreceptor was fabricated and characterized for fluorescence-based biomarker detection, and then modified with poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) to enhance its electrochemical performance. This technology is a potential candidate for real-time biomarker monitoring in complex biological fluids, as its porous network filters out large interfering material. PEGDA-AA hydrogels were synthetized and characterized using TBO, FTIR spectroscopy and swelling tests. The successful encapsulation of a DNA bioreceptor was verified through fluorescence measurements of its fluorescein amidite (FAM)-labeled complementary strand. Rheological studies revealed rapid photopolymerization kinetics and high compressibility. Integration with PEDOT:PSS conducting polymer yielded a highly conductive material (4.6 S/cm, via EIS), demonstrating the platform's potential for the development of highly specific electrochemical biosensors. Future research will focus on testing the biosensing response of PEGDA-AA-PEDOT:PSS hydrogel through electrochemical detection of different neurological biomarkers in biological fluids, as well as its implementation in wearable formats.

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Reference number: T03-5

IDENTIFICATION OF CFS/ME BIOMARKERS WITH CITE-SEQUENCING TECHNOLOGY

Chronic Fatigue Syndrome or Myalgic Encephalomyelitis (CFS/ME) is a debilitating disorder, often considered a neurological autoimmune condition; although its etiopathogenesis remains poorly understood. It affects approximately 0.1-0.8% of the population, with severe cases leading to housebound or even bedbound patients, meaningfully impairing quality of life. Previous studies have reported alterations in the peripheral blood mononuclear cell (PBMC) ratio, and single-cell transcriptomic analyses have identified many potential biomarkers, particularly differentially expressed genes (DEG) on monocytes.

In this study, we analyzed eight samples, including four CFS/ME patients and four controls not diagnosed with CFS/ME, who were brothers or sisters of the patients. Monocytes were isolated and processed to run cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq). This new single-cell approach enables simultaneous transcriptomic and proteomic profiling through an antibody-based panel. Therefore, it allows a deeper analysis and integrates a functional insight based on the correlation between transcripts and proteins, strengthening the results. Using the processed transcriptomic and proteomic data, we performed exhaustive bioinformatic analysis to identify clusters, pathways, mechanisms and biomarkers dysregulated in the cells of the CFS patients. On the conference, we will present a more complete analysis of the omics data we acquired. For future research, our results may be helpful in order to understand etiopathogenesis and look for a treatment.

Authors

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Reference number: T03-6

DECIPHERING THE ROLE OF THE IRF5-P2X4 SIGNALING PATHWAY IN MULTIPLE SCLEROSIS PATIENTS

Efficient clearance of myelin debris by microglia and infiltrating macrophages is essential for remyelination during multiple sclerosis (MS) progression. Previous studies have shown that activation of the purinergic receptor P2X4 enhances myelin phagocytosis. In parallel, the transcription factor Irf5, a microglial risk gene linked to MS, promotes a reactive $P2X4^{+}$ phenotype and is critical for myelin processing. IRF5 also regulates

myelin-derived cholesterol homeostasis, which is essential for the microglial regenerative response. Despite the growing evidence supporting the relevance of the IRF5-P2X4 axis in MS, its specific role remains unclear. We aimed to determine whether the IRF5-P2X4 signaling pathway modulates microglial lipid metabolism and to evaluate its clinical relevance in MS patients. Under demyelination-like conditions, Irf5$^{-/-}$ cultured microglia showed downregulation of Ch25h, an interferon-responsive gene involved in cholesterol metabolism, together with reduced expression of the cholesterol transporters Abca1 and Abcg1, leading to cholesterol crystal accumulation. Reanalysis of publicly available scRNA-seq datasets revealed that IRF5 expression is largely restricted to microglia and increased in MS patients, whereas P2RX4 expression was elevated in microglia and other central nervous system cell populations during disease progression. To validate these findings in human samples, plasma cytokines, neurodegeneration (NfL), and astrocytosis-related biomarker levels were analyzed using Luminex and SIMOA technologies in an MS patient cohort. In addition, qPCR analysis demonstrated altered IRF5 and P2RX4 expression in peripheral blood mononuclear cells (PBMCs). Finally, mixed-effects linear model analysis identified IRF5 expression as a predictor of disease progression based on longitudinal EDSS evaluation. Together, these findings highlight the IRF5–P2X4 signaling axis as a key regulator of microglial function and a promising therapeutic target in MS.

Authors

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Reference number: T03-7

DYNAMIC MODE DECOMPOSITION FOR DISCOVERING BIOMECHANICAL BIOMARKERS IN PARKINSONIAN MICE WITH GAIT ALTERATIONS.

Freezing of gait is a common symptom of Parkinson's disease patients, leading to movement impairment and falls, reducing greatly their quality of life. Understanding the spatiotemporal dynamics that govern healthy and parkinsonian gaits may lead to better diagnosis and better evaluation of the effectiveness of treatments. In this work, we used a mouse model of Parkinson's disease to investigate potential gait alterations. Our aim was to identify biomechanical biomarkers that could serve to identify deficits that may reflect early or progressive features of the disease and potentially drive smart neuromodulation systems. We hypothesize that gait in healthy rodents obey oscillatory patterns of relatively constant frequency while parkinsonian ones show additional interfering motor patterns impeding normal behavior. Using the data-driven method called dynamic mode decomposition (DMD), a dimensionality reduction technique, we are able to isolate and describe the main movement patterns involved in walking mice as damped/driven oscillators. With this analysis, a deeper understanding of the motor patterns can be reached and may lead to the discovery of visual spatiotemporal biomarkers for the disease.

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Reference number: T03-8

TRANSLATING HUMAN RESILIENCE MECHANISMS INTO THERAPEUTIC STRATEGIES USING BRAIN ORGANOIDS THAT RECAPITULATE ALZHEIMER'S DISEASE.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of amyloid-beta plaques and tau neurofibrillary tangles. It is the leading cause of dementia worldwide and results in progressive cognitive decline and irreversible neuronal loss. Although amyloid and tau pathology define AD, neuropathological burden alone does not fully explain cognitive impairment. Notably, approximately one-third of older adults show high levels of AD neuropathology at autopsy while remaining cognitively intact during life. This dissociation suggests that endogenous protective mechanisms can promote resilience to AD pathology and preserve cognitive function despite substantial disease burden.

The aim of our study is to translate these human resilience mechanisms into novel therapeutic strategies. To achieve this, we are developing two multidisciplinary and complementary approaches: (i) computational methods to identify molecular targets associated with resilience, and (ii) a physiologically relevant 3D in vitro platform using AD patient-derived brain organoids to validate therapeutic candidates. This strategy seeks to establish a clinically relevant model that recapitulates key aspects of AD pathology and can be used for future high-throughput drug screening. Patient-derived brain organoids therefore represent a cutting-edge platform for the discovery and preclinical validation of new therapeutic approaches for AD.

Using computational approaches that integrate postmortem brain multi-omics, neuropathology, neuroimaging, clinical, and cognitive data, we aim to identify molecular mechanisms that promote resilience and nominate candidate therapeutic targets. We are developing advanced analytical frameworks and exploring quantum-inspired algorithms to enhance computational capacity and target prioritization. Our initial computational estimates converge on several resilience-associated mechanisms, including reduced inflammatory signaling, lower glia-mediated synaptic engulfment, preservation of cellular metabolism, and the presence of microglial states linked to cognitive preservation.

In parallel, during the last year we have developed immunocompetent cerebral organoids that reproduce key AD-related pathological and neuroimmune features. Standard cerebral organoids contain neurons and astrocytes but often lack endogenous microglia, limiting their ability to model neuroimmune response. To generate a more biomimetic model, we derived patient-matched microglia through yolk-sac-like differentiation from the same induced pluripotent stem cell lines used to generate the cerebral organoids. These cell populations were incorporated into the organoid system to create a multilineage model containing neurons, astrocytes, and microglia.

The resulting organoids have been validated using immunohistochemistry to assess cellular composition, structural organization, and integration of the different cell types. In addition, key AD-related pathological hallmarks have been quantified to evaluate disease fidelity and determine whether the model captures relevant features of patient-specific pathology. This multilineage organoid platform will allow us to systematically investigate the neuroimmune response that may contribute to cognitive resilience and to test therapeutic strategies aimed at inducing resilience-like states.

In future studies, once the platform is fully optimized, we will evaluate therapeutic approaches designed to modulate these resilience mechanisms, including CRISPR-based gene editing, antisense oligonucleotides, and other targeted interventions. Ultimately, this work aims to create a personalized medicine platform for the preclinical validation of resilience-promoting therapies in AD.

Authors

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Reference number: T03-10

WHOLE-EXOME SEQUENCING–BASED COMPUTATIONAL PROFILING REVEALS REDUCED ECCDNA BURDEN AND DISTINCT GENOMIC INSTABILITY PATTERNS IN IDH-MUTANT GLIOBLASTOMA

Glioblastoma (GBM) is the most aggressive form of glioma, with a one-year overall survival rate below 5%. Isocitrate dehydrogenase (IDH) mutation status is a well-established prognostic biomarker in glioblastoma, with IDH-mutant (IDHmut) tumors generally associated with longer survival and a less aggressive phenotype compared with IDH wild-type (IDHwt) GBMs. Another hallmark of aggressive tumor behavior is the presence of extrachromosomal circular DNA (eccDNA), non-plasmid circular DNA molecules associated with genomic instability and tumor evolution. EccDNA is commonly identified using Circle-seq or whole-genome sequencing (WGS) data.
Here, we present a bioinformatics pipeline for the identification of eccDNA signatures from whole-exome sequencing (WES) data, providing a cost-effective alternative for eccDNA detection and analysis. Using this workflow, we analyzed WES data from 13 GBM tumors to detect eccDNA burden and somatic mutations. Our results demonstrate that WES can effectively identify eccDNA-associated genomic instability patterns in GBM. Specifically, IDHmut samples displayed only 74 eccDNA signals compared with approximately 1,700 detected in IDHwt tumors. Similarly, IDHmut samples harbored only 24 somatic mutations, whereas IDHwt tumors presented nearly 1,500 mutations. These observations support the hypothesis that the markedly lower number of eccDNA molecules detected in IDHmut tumors reflects a significantly reduced level of genomic instability compared with IDHwt GBMs. Because eccDNA formation has been associated with DNA damage, chromosomal rearrangements, and oncogene amplification, the limited eccDNA burden observed in IDHmut samples may contribute to their less aggressive biological behavior and improved clinical prognosis. In contrast, the accumulation of eccDNA in IDHwt tumors may represent an adaptive mechanism that promotes rapid evolution, and therapeutic resistance. Together, these findings suggest that eccDNA abundance could serve not only as a biomarker of genomic instability, but also as a potential indicator of glioblastoma aggressive phenotype linked to IDH status.

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Reference number: T03-11

CIRCUIT-SPECIFIC ELECTROPHYSIOLOGICAL ALTERATIONS IN THE LOCUS COERULEUS DURING PRODROMAL PARKINSON'S DISEASE.

Abstract:
Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder associated with non-motor symptoms that often emerge during prodromal stages. The locus coeruleus (LC), the main noradrenergic nucleus of the brain, is among the regions affected early, and its dysfunction and α-synuclein (α-syn)-associated pathology have been linked to early emotional and cognitive disturbances. Despite being small, the LC contains distinct neuronal subpopulations that project to specific brain regions, including the amygdala and the hippocampus (HP), which play key roles in emotional processing and cognition, respectively. However, the functional impact of early α-syn pathology on specific LC-limbic circuits remains unknown. In this study, we investigated whether α-syn overexpression in the LC alters the electrophysiological properties of noradrenergic LC neurons projecting to the central amygdala (CeA) and HP, as potential early mechanisms underlying non-motor symptoms in PD. Using a prodromal PD mouse model with viral-mediated human α-syn overexpression in the LC, LC-CeA and LC-HP neurons were retrogradely labeled with retrobeads injected into their respective target regions. Ex vivo electrophysiological recordings were performed in brainstem slices to assess intrinsic membrane properties and neuronal excitability in these projection-defined LC neurons. Preliminary results reveal subtle alterations in electrophysiological properties of LC-CeA and LC-HP neurons in α-syn– overexpressing mice, with potential sex-dependent and projection-specific differences. These findings suggest that α-syn accumulation may selectively affect specific LC neuronal subpopulations, supporting the hypothesis that early circuit-level dysfunction contributes to the emergence of emotional and cognitive non-motor symptoms during initial stages of PD.
Keywords: Parkinson's disease, prodromal stage, locus coeruleus, noradrenergic neurons, α-synuclein, limbic circuits, central amygdala, hippocampus.
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Reference number: T03-12

2D AND 3D HUMAN IN VITRO MODELS TO INVESTIGATE CALCIUM DYSREGULATION AND FUNCTIONAL DECLINE IN NEUROMUSCULAR DISEASES

Duchenne Muscular Dystrophy (DMD) is characterized not only by progressive muscle degeneration but also by profound alterations in calcium homeostasis and excitation–contraction coupling, which contribute to muscle weakness and fatigue. Investigating these functional impairments in humanrelevant systems remains challenging, as traditional animal models often fail to accurately reproduce human neuromuscular pathology. Human-based New Approach Methodologies (NAMs), including engineered

3D muscle tissues, provide physiologically relevant platforms to study disease-associated alterations in calcium dynamics and contractile function.
In this study, we used immortalized human myotubes to investigate functional deficits associated with DMD and evaluate the effects of the novel calcium modulator MP-001. In 2D impedance-based assays, DMD myotubes consistently displayed impaired myogenic behavior. While MP-001 induced only a modest improvement in the myogenic phenotype, functional calcium imaging revealed a significant restoration of Ca^{2+} homeostasis following treatment, suggesting a targeted effect on excitation–contraction coupling mechanisms. To further investigate disease-associated functional decline in a more physiological environment, we developed 3D fibrin-based muscle constructs. Preliminary experiments demonstrated that higher cell densities significantly improved tissue integrity and
functional performance. Contractility analyses revealed marked deficits in DMD constructs, including increased susceptibility to fatigue, consistent with the progressive neuromuscular impairment observed in DMD patients.
Our findings highlight the importance of integrating functional readouts, such as Ca^{2+} dynamics and contractility, into human neuromuscular NAMs for research. While 2D systems are suitable for highthroughput phenotypic screening, 3D engineered muscle models provide the physiological maturity required to investigate excitation–contraction coupling and fatigue-related dysfunction. Together, these platforms offer valuable tools for studying disease pathophysiology and evaluating therapies targeting functional decline in neuromuscular disorders.

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Reference number: T03-13

EARLY FUNCTIONAL SIGNATURE OF MOTOR NEURON COLLAPSE IN HUMAN IN VITRO ALS MODELS

Introduction:
The investigation of Amyotrophic Lateral Sclerosis (ALS) requires human in vitro models capable of faithfully recapitulating spinal motor neuron physiology. While TDP-43 nuclear loss-of-function and the emergence of cryptic exons are established pathological hallmarks, they often represent terminal stages of the disease. This temporal lag obscures the identification of early pathogenic mechanisms and potential reversible therapeutic targets.
Objectives:
In this study, we developed and validated the pNAIL protocol (transduction of NGN2, ISL1, and LHX3) against generic induction models (pUNA) to establish a high-fidelity platform. Subsequently, we utilized this model to characterize the functional impact of the TDP-43 Q331K mutation during prodromal stages.
Methodology:
Human iPSC reprogramming techniques were employed in combination with inducible lentiviral vectors. Lineage fidelity was assessed through transcriptomic profiling and exhaustive electrophysiological characterization using Micro-Electrode Arrays (MEAs)We performed a multiscale analysis of evoked potentials, local field potentials (LFPs), and higher-order network dynamics, such as Phase-Amplitude Coupling (PAC).

Results:
The pNAIL model demonstrated significant superiority in functional maturity compared to the generic model. While pUNA networks operate in high-bandwidth (cortical-like) regimes, pNAIL networks exhibit rhythmic precision, stability, and a hierarchical organization—measured by robust PAC—that aligns with spinal motor neuron physiology.
In this high-fidelity context, the Q331K mutation revealed a biphasic functional trajectory: an initial hyperactivity sustained by a metabolic switch toward glycolysis, followed by homeostatic collapse. Notably, this phenotype of vulnerability and calcium dysregulation manifests in the absence of cryptic exons, suggesting that bioenergetic dysfunction precedes classical splicing defects.
Conclusions:
Our findings consolidate the pNAIL protocol as a robust platform for ALS modeling. The detection of early functional alterations linked to the Q331K mutation underscores the importance of integrating electrophysiological and metabolic metrics to identify therapeutic intervention windows prior to irreversible cellular collapse.

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MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION AND GLIOTRANSMISSION BY CB1 RECEPTORS IN THE LATERAL HABENULA

The habenular complex (HbCpx) is a highly conserved brain structure crucially involved in the (patho)biology of emotional states. This nucleus is composed of lateral (LHb) and medial (MHb) and habenular subnuclei that display different anatomical connectivity and contain heterogeneous populations of neurons and astroglial cells. Hyperactivity of the LHb has been consistently associated with depression and deficient emotional control through mechanisms that involve increased neuronal activity, adaptations in synaptic plasticity, and aberrant astrocyte-neuron interactions.
Endocannabinoids modulate brain function and behavior by targeting cannabinoid CB1 receptors (CB1R) in neurons and astroglial cells. These receptor populations fine-tune synaptic transmission and astrocyte-to-neuron communication, respectively, and critically regulate motivation and emotional behavior in physiological conditions. Nevertheless, the functional roles of CB1R in synaptic and astrocyte-mediated signaling within the LHb remain scantly known.
Here, we investigated the effect of CB1R activation on excitatory synaptic transmission and astrocytic calcium signaling in the LHb of adult male and female mice. Whole-cell patch-clamp recordings from LHb neurons were used to evaluate spontaneous and evoked excitatory postsynaptic currents (EPSCs) following 5 min exposure to the CB1R agonist WIN55,212-2. Bath application of the cannabinoid agonist did not modify the frequency, amplitude, width, rise time or decay time of sEPSCs in either male or female mice. In contrast, WIN55,212-2 increased the amplitude of eEPSCs selectively in female mice. Ex vivo imaging experiments following injection of GFAP-GCaMP6f showed that LHb astrocytes respond to brief applications of WIN with intracellular calcium elevations of increased frequency. Together, these preliminary findings point to a sex-dependent

regulation of LHb excitatory transmission and gliotransmission by CB1R. Although the functional implications of CB1R-mediated astrocytic calcium signaling on LHb neurons remains to be demonstrated, our results support the possibility that astroglial mechanisms contribute to cannabinoid-dependent modulation of habenular activity, with potential implications in the control of emotional behavior both in physiological and pathological conditions.

Keywords: lateral habenula, CB1 receptors, endocannabinoids, astrocytes, synaptic transmission, gliotransmission.

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Reference number: T03-15

DEVELOPMENT OF A CHRONIC IN VIVO ELECTROPHYSIOLOGICAL PARADIGM FOR MONITORING HYPERACUTE CEREBRAL ISCHEMIA IN AWAKE MICE

Stroke is a leading cause of mortality and long-lasting disability worldwide. However, the earliest pathophysiological events, occurring within minutes to first hours after ischemic onset, remain poorly understood. In this study, we present the technical development and preliminary validation of a novel paradigm designed for real-time in vivo characterization of neuronal activity in hyperacute cerebral ischemia in awake mice. This method integrates phototrombic stroke induction with chronic electrophysiological recording, enabling simultaneous monitoring of neuronal dynamics during the hyperacute phase of ischemic stroke.

Custom microdrives based on the Kubie design were developed to combine a 16-channel tetrode array with an integrated optical fiber for focal photothrombosis. The drive consisted of four tetrodes; each made from four twisted 25-µm Teflon-insulated nichrome wires. A 200-µm diameter, 10-mm fiber cannula positioned adjacent to the recording site enabled localized Rose Bengal photothrombotic induction using a 560-nm laser while preserving the ability to record neuronal activity near the ischemic core. Signals were acquired in awake freely moving mice before, during and up to 24 hours after phototrombic stroke.

This methodological approach enables longitudinal recordings from the first seconds following ischemic induction through subsequent stages of stroke progression. Preliminary data demonstrate that this strategy can induce focal cortical ischemia while maintaining stable electrophysiological recordings throughout the procedure. Early analysis reveals dynamic alterations in neuronal firing patterns, including changes in spike amplitude and firing frequency following ischemic insultstroke. These findings support the feasibility of this strategy for continuous monitoring of neuronal dynamics from the onset of ischemic insult through hours post-ischemia. Moreover, they provide a foundation for future studies investigating the temporal mechanism underlying brain injury across the hyperacute, acute, and subacute phases of stroke.

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Reference number: T03-16

TACKLING MITOCHONDRIA–ER COMMUNICATION TO MITIGATE NEURODEGENERATIVE STRESS

Neurodegenerative disorders are characterized by the early disruption of fundamental cellular processes, including calcium homeostasis, lipid metabolism, organelle quality control, and stress adaptation, all of which critically depend on mitochondria–endoplasmic reticulum (ER) communication. Increasing evidence indicates that alterations in mitochondria–ER communication occur prior to the appearance of classical pathological hallmarks, positioning these interactions as key regulators of neuronal vulnerability and early cellular dysfunction, as well as promising therapeutic targets. In this context, we present MERLIN (Mitochondria–ER Length Indicator Nanosensor), a reversible BRET-based biosensor designed to monitor mitochondria–ER communication dynamics in real time and facilitate the identification of small-molecule modulators of inter-organelle signaling. MERLIN provides a rapid, cost-effective, and experimentally accessible platform for detecting changes in organelle proximity and was successfully adapted for high-throughput screening applications. Using this approach, we identified several compounds capable of modulating the MERLIN signal in a concentration-dependent manner without affecting cell viability, consistent with selective effects on mitochondria–ER communication dynamics and potential therapeutic relevance. In parallel, we combined APEX2-based proximity labeling with proteomic approaches to characterize the molecular composition of mitochondria–ER contact sites and identify regulatory factors associated with these interfaces. Using this strategy, we identified mitochondrial carrier homolog 2 (MTCH2), a MERCS-associated protein, as a modulator of mitochondria–ER communication and inflammatory responses during cell death, highlighting the contribution of inter-organelle signaling dynamics to neurodegenerative stress responses.

Authors

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DECNEFSIMULATOR: A MODULAR, INTERPRETABLE FRAMEWORK FOR DECODED NEUROFEEDBACK SIMULATION USING GENERATIVE MODELS

Decoded Neurofeedback (DecNef) is a flourishing non-invasive approach to brain modulation with wide-ranging applications in neuromedicine and cognitive neuroscience. However, progress in DecNef research remains constrained by subject-dependent learning variability, reliance on indirect measures to quantify progress, and the high cost and time demands of experimentation.

We present DecNefSimulator, a modular and interpretable simulation framework that formalizes DecNef as a machine learning problem. Beyond providing a virtual laboratory, DecNefSimulator enables researchers to model, analyze and understand neurofeedback dynamics. Using latent variable generative models as simulated participants, DecNefSimulator allows direct observation of internal cognitive states and

systematic evaluation of how different protocol designs and subject characteristics influence learning.
We demonstrate how this approach can (i) reproduce empirical phenomena of DecNef learning, (ii) identify conditions under which DecNef feedback fails to induce learning, and (iii) guide the design of more robust and reliable DecNef protocols in silicoqu before human implementation.
In summary, DecNefSimulator bridges computational modeling and cognitive neuroscience, offering a principled foundation for methodological innovation, robust protocol design, and ultimately, a deeper understanding of DecNef-based brain modulation.

Authors
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Reference number: T03-18

SEQUENCE-TO-FUNCTION AI MODELS REVEAL THE HIDDEN REGULATORY ARCHITECTURE OF ALS.

Sporadic Amyotrophic Lateral Sclerosis (ALS) is driven by a complex non-coding genetic architecture. To decode its regulatory mechanisms, we deployed AlphaGenome, a deep-learning sequence-to-function model, analyzing susceptibility loci identified through Genome-Wide Association Studies (GWAS) and Summary-data-based Mendelian Randomization (SMR) datasets from the Project MinE initiative. By computing a Multidimensional Damage Score (MDS) derived from sequence-based multi-omic predictions (ATAC, CAGE, and RNA-seq and splicing), we prioritized a refined cohort of 268 highly disruptive polymorphisms. Our analysis reveals a profound structural paradigm shift: while classical prioritization relies on GWAS statistical risk associations—which favor coding or intronic loci—true molecular disruption concentrates disproportionately within distal intergenic regulatory elements. Spatial mapping of these variants uncovered a "dual-hit" pathobiology, linking intrinsic CNS vesicular transport defects with profound systemic immune dysregulation, notably implicating altered immune proteostasis. Cross-validation confirmed established bulk-tissue targets but revealed that over 70% of highly disruptive regulatory interactions are novel "SMR orphans." These context-specific causal links, exemplified by pleiotropic enhancer-hijacking (e.g., rs631312 to CX3CR1/MYRIP) and UPP1 chromatin ablation in T-cells, remain invisible to standard transcriptomics. Furthermore, in silico simulation of the C9orf72 hexanucleotide repeat expansion precisely recapitulated its clinical pathogenic threshold and unmasked a length-dependent pleiotropic toxicity: immune-specific promoter silencing coupled with CNS-specific enhancer hijacking of distant targets like LINGO2. By bridging raw GWAS signals to precise 3D-regulatory syntax, this AI-driven framework provides a powerful tool to unmask the missing functional heritability of sporadic ALS and other complex disorders with a substantial genetic burden.

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Reference number: T03-19

IN VIVO MULTIMODAL IMAGING OF PURINERGIC P2X7 RECEPTORS IN ISCHEMIC RESPONSE AFTER AN EXPERIMENTAL MODEL OF STROKE IN RATS

Ischemic stroke leads to elevated extracellular adenosine 5'-triphosphate levels, highlighting the relevance of purinergic receptors, particularly the P2X7 receptor (P2X7R), as potential therapeutic targets1. As their diagnostic and therapeutic value remains poorly characterized, this study aimed to longitudinally assess in vivo P2X7R expression in healthy and ischemic rats and to investigate its role in ischemic response using non-invasive imaging approaches.
Transient middle cerebral artery occlusion (tMCAO) was induced for 75 minutes, followed by magnetic resonance imaging (MRI) and positron emission tomography (PET) using the selective P2X7R radiotracer [18F]JNJ-644137392 at baseline and up to one month post-ischemia. Complementary ex vivo analyses were performed to further characterize receptor expression. To evaluate the functional contribution of P2X7R, rats were administered the selective P2X7R antagonist A804598 (2.5 mg/kg) during the first week after ischemia. MRI and PET imaging were conducted on days 3 and 7 to assess neuroinflammation ([18F]DPA-714), matrix metalloproteinase activation ([18F]BR-351), and oxidative stress ([18F]FSPG). Under physiological conditions, P2X7R expression was minimal across brain regions. MRI revealed a cortico-striatal infarction accompanied by a progressive increase in PET-[18F]JNJ-64413739 signal up to day 7, followed by a subsequent decline in both cortex and striatum. This pattern was consistent with an increased presence of CD11b+/P2X7R+ microglia/macrophages within the ischemic area. Regarding the role of P2X7R in the ischemic stroke, lower [18F]FSPG-tracer uptake was observed in the treated group on day 3, followed by a lower uptake of [18F]DPA-714 and a higher uptake of [18F]BR-351 on day 7. Overall, these findings demonstrate that P2X7R plays a key role in post-ischemic pathophysiology, supporting its potential as a therapeutic target.

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PHENOTYPIC DIVERSITY DETERMINES THE THERAPEUTIC RESPONSE TO FKBP12-TARGETING RYR1 STABILIZERS IN RYR1-RELATED MYOPATHIES

RyR1-related myopathies (RyR1-RM) are heterogeneous neuromuscular disorders in which distinct RyR1 variants produce different calcium-handling and excitation-contraction coupling defects. Here, we evaluated whether mutation-specific phenotypes determine the response to FKBP12-targeting RyR1 stabilizers, focusing on MP-001. Using RyR1-expressing HEK293 cells, patient-derived transdifferentiated myotubes, and immortalized myotubes, we combined calcium imaging, ER/SR Ca^{2+} measurements, single-channel recordings, and electrical stimulation. MP-001 improved RyR1 function in gain-of-function/leaky variants associated with FKBP12 dissociation, restoring calcium homeostasis and excitation-contraction coupling. In contrast, the loss-of-function

G4638V variant showed SR Ca^{2+} overload, impaired electrically evoked Ca^{2+} responses, SR stress, and no therapeutic benefit. These results indicate that FKBP12-RyR1 stabilizers are effective only in selected RyR1-RM phenotypes. Functional stratification of RyR1 variants will therefore be essential for future preclinical and clinical development of precision therapies for neuromuscular RyR1-related diseases.

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