Poster 1

Multi-modal spatial profiling of a chimpanzee frontal pole

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The disproportionate expansion of the human association cortex following the divergence of human and chimpanzee lineages was likely accompanied by substantial spatial reorganisation of molecular and cellular features. Although cross-species differences in bulk transcriptomic profiles and cell type proportions in human and non-human primate brains have been characterised to a certain extent, differences in the spatial organisation of cell types and molecular signatures, and how these relate to genetic and neuroanatomical divergence, remain poorly understood. We previously generated the first spatial transcriptomic dataset (Visium) from a great ape brain, using fresh-frozen *post mortem* frontal pole tissue from an adult female chimpanzee. Here, we build on this resource and aim to integrate our existing spatial transcriptomic data with spatial lipidomics and iron accumulation data obtained using matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry and X-ray fluorescence (XRF) imaging. By doing so, we aim to explore putative molecular links between the spatial organisation of gene expression, lipid metabolism, and iron accumulation patterns. To date, we have successfully applied each of these three techniques independently to different brain samples. In this pilot study, our goal is to generate and integrate all three data modalities from consecutive sections of a single fresh-frozen frontal pole chimpanzee brain sample, enabling us to investigate cross-modal spatial associations. Overall, our work i) provides an integrative multi-modal spatial analysis pipeline for investigating the molecular underpinnings of the chimpanzee frontal pole, and ii) represents a first step towards spatially resolved multi-modal characterisation of great ape brains.

Poster 2

The role of phosphorylation in evolutionary neurodevelopmental divergence

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Phosphorylation is the most prevalent of the post-translational modifications (PTMs) (Bradley, 2022) and the most common PTM identified in sites that have a missense variant in modern humans compared to ancestral hominids (Pravata et al., 2025). We are exploring whether changes in protein phosphorylation might have contributed to evolutionary divergences in brain development. We employed mass spectrometry and Fe-IMAC for assessing the total proteome and phosphoproteomic landscape of induced pluripotent stem cell (iPSC)-derived neural progenitor cells (NPCs) from human and chimpanzee lines. Our results reveal that 73.5% of the total NPC proteome was shared between species, and approximately 35% of the identified proteins were phosphorylated in both species. Out of 4,475 phosphorylated proteins, 64.1% were phosphorylated in both species, 19.6% were unique to humans and 16.3% were unique to chimpanzees. To investigate if there are key kinases that are more present or active in one of the species, an *in silico* kinase-phosphosite prediction was performed. Among the top 50 predicted kinases, 34 were different between the species. 89% of human phosphosites and 98% of chimp phosphosites were conserved between the species, indicating that changes in phosphorylation are not due to phosphosite mutations. Preliminary data indicates that, although phosphorylation rates and kinase presence are similar between species, differences in which proteins are phosphorylated could be related to evolutionary divergences such as brain size and language development. Next steps include comparing presence and activity of candidate kinases, as well as investigating how phosphorylation of specific brain development-related proteins impact the function of these proteins across species.

Poster 3

Neuroanatomical and Chemoarchitectural Characterization of the Human Pretectal Region during the Second Trimester and Childhood

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The human pretectum (PRT) is a brain part situated in the diencephalon-mesencephalic junction. It plays a vital role in controlling the brain's visual and oculomotor systems like pupillary light reflex, and has extensive connectivity patterns with subcortical visual centers. Despite its critical functional significance, human pretectum is poorly differentiated, and it is often referred integrally to describe the pupillary light pathways and the Optokinetic Nystagmus response. As the entire pretectum is not well-characterized, this study addresses the critical gap by providing a systematic characterization of the topographical, cytoarchitectural, and chemoarchitectural organization of the fetal human PRT during the second trimester [14 - 25 gestational weeks (GW)], along with a 9-year-old specimen. Utilizing high resolution histological datasets from coronally sectioned 5 specimens, and a multi-marker immunohistochemical panel including calretinin, calbindin, parvalbumin, neuropeptide Y (NPY), and Glial Fibillary Acidic Protein (GFAP), we delineated nine distinct nuclei as early as 14 GW: the nucleus of the optic tract (NOT), the anterior (APN), posterior (PPN), olivary (OPN), and medial (MPN) pretectal nuclei, the central tegmental area (CTA), the nucleus of the posterior commissure (NPC) and its magnocellular nucleus (MCPC). In Nissl, the APN exhibited a cell sparse pattern. In contrast, the CTA showed dense cellularity. The OPN was uniquely distinguished by a whirlpool-like cellular arrangement, and the PPN is ventral and medial to the OPN and NOT. MPN is the smallest PRT nucleus, located lateral to the pineal gland and NOT is located between the MPN and OPN. The MCPC is characterized by a sparse matrix with large cells. NPC is a cell-sparse region. By 19 GW, APN lacked calretinin/calbindin reactivity, and CTA showed strong parvalbumin positivity. The PPN and MPN demonstrated clear parvalbumin immunoreactivity and OPN lacked parvalbumin reactivity. NPY expression was observed in labeled neurons in APN, and NPY-ir fibers in other nuclei, at 25 GW and in the 9-year-old specimen. The progressive maturation of the astrocytic scaffold across the second trimester was revealed by GFAP staining. By integrating these data, this work delineates anatomically heterogeneous developing human pretectum providing insights for understanding its maturation and functional significance in neurodevelopment.

Poster 4

Human-chimpanzee tetraploid system uncovers transcription factors underlying species-specific changes in neurogenesis

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A major challenge in human evolutionary biology is to pinpoint genetic differences that underlie human-specific traits, such as brain size. We used human-chimpanzee tetraploid cells to distinguish gene expression changes due to *cis*-acting sequence variants that change local gene regulation, from *trans*-regulated expression changes due to species differences in the cellular environment in neural progenitor cells (NPCs). Examination of both *cis* and *trans* changes identified *cis*-regulated, human-specific increases in expression of the transcription factors *FOSL2* and *MAZ*, and a corresponding increase in FOSL2 and MAZ binding at existing binding sites genome-wide. CRISPR inhibition of *FOSL2* and *MAZ* in human NPCs activated gene sets associated with neuron differentiation and maturation, suggesting that increased expression of these transcription factors in human NPCs may prolong neurogenesis. To test this hypothesis, we overexpressed *FOSL2* and *MAZ* in mouse cortex via *in utero* electroporation at E13.5. We found that cells overexpressing *FOSL2* or *MAZ* are more likely to retain neural progenitor identity and less likely to produce neurons at E15.5, suggesting that increased *FOSL2* and *MAZ* expression promotes NPC self-renewal and may ultimately contribute to prolonged neurogenesis in the human brain. This study identifies *cis*-acting genomic changes that lead to downstream *trans* gene regulatory effects to contribute to human-specific differences in brain development, providing a framework for the discovery of genetic differences underlying human traits.

Poster 5

Input-Output Circuit Mapping to the Higher-Order Relay Laterodorsal Nucleus of the Thalamus in Mice

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The thalamus, beyond relaying sensory information to the cortex, plays a key role in attention, memory, and cognition. The anterior nuclei of the thalamus (ATN) serve as a central hub of the limbic system, a network long known to be involved in emotion and memory. Over the past two decades, studies have shown that anterodorsal (AD) and laterodorsal (LD) nuclei, both components of ATN, play a key role in the integration of head direction system and visual-landmark information, a computation that ensures the stability of the visuospatial map. In contrast to the AD nucleus, anatomical and functional features of the LD are not well defined.

Here, we analyzed the architecture and carried out a brain-wide mapping of the input-output motifs in the mouse laterodorsal (LD) nucleus at micropopulation level using anterograde and retrograde labeling methods. Specifically, we mapped and quantified the sources of cortical and subcortical input to different LD subregions and compared the distribution of their axons.

We have demonstrated that LD receives its primary ascending afferents from visual-related structures, including the pretectum, superior colliculus and pregeniculate nucleus. We also found that LD neurons innervate the cingulate, retrosplenial, visual and parahipocampal areas with a specific projection motif.

Altogether, these data suggest that subcortical inputs to LD could bring visual-landmark information to the extended hippocampal system and to the retrosplenial cortex, two areas critically involved in the computation of the visuospatial map.

Poster 6

Investigation of ECM-mediated Region-specific Interactions between Neural Progenitors and Microglia during Telencephalic Development

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Microglia is a unique cell type that has been studied extensively in the adult brain, due to the implication in disease and physiological brain homeostasis. Deriving from the yolk sac, microglia is one of the first glial cell types to colonize the developing neuroepithelia, preceding the peak of neurogenesis in the developing telencephalon (Menassa & Gomez-Nicola, 2018). Previous studies have shown that during early development of the telencephalon, microglia appear to colonize heavily the proliferative zones of the primate cortex (Penna et al., 2021). Microglia shows one of the highest regional heterogeneity in the brain, on a morphological, transcriptional and proteomic level. Only recently more attention has been directed toward the role of microglia in brain development (Yu et al., 2025, Lawrence et al., 2024, Park et al., 2023).

We therefore ask if microglia show similar heterogeneity during development, specifically during telencephalic development and what are the effects of the presence of developing microglia in the neural progenitor pools of different regions of the developing neuroepithelia. We have developed a model system in which we can incorporate hematopoietic progenitor cells (HPCs) derived from human induced pluripotent stem cells (iPSCs) in patterned cerebral organoids of two different identities: dorsally patterned and ventrally patterned cerebral organoids. HPCs are then differentiated to

Poster 7

Exploring the miRNA landscape of primate astrocyte extracellular vesicles

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Astrocytes have undergone significant morphological changes during evolution of mammals. Human astrocytes are larger and more complex than those of non-human primates (NHPs), whereas primate astrocytes exhibit greater diversity than rodent astrocytes. There is increasing evidence suggesting that astrocyte-secreted factors contribute to the advanced functional properties of the human brain. In our previous work, we observed increased activation of genes associated with extracellular exosomes in human astrocytes compared with those of NHP, marked by higher production of extracellular vesicles (EV) by human (iPSC)-derived astrocytes (iAstrocytes).

EVs facilitate intercellular communication in the brain by transferring molecular cargo, including microRNAs (miRNAs), which can influence neuronal function. Evolutionary changes in EV composition may therefore represent an additional layer of regulation in brain development. However, the evolutionary changes in astrocyte-derived EV cargo remains poorly characterized.

In this study, we profiled miRNAs carried by EVs derived from human and chimpanzee induced pluripotent stem cell iAstrocytes in order to explore evolutionary diUerences in EV content. We identified diUerentially expressed miRNAs based on sequencing and screened them for potential targets involved in early neuronal development. We then characterized the impact of selected miRNAs on iPSCs-derived human neurons. Preliminary analyses indicate implications for the rearrangements of the extracellular matrix, which is critical for orchestrating the shape and timing of early neuronal and synaptic development. Ongoing work combines iPSC models, microscopy and next-generation sequencing to improve our understanding of how these astrocytic miRNAs had advanced human brain development.

Poster 8

Role of Reelin in vertebrate pallial development and evolution

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Mammalian cerebral cortex development is a multistep process leading to the formation of layered pallial structures essential for higher-order brain functions. A key factor in this process is the secreted glycoprotein Reelin.

While it is well established that Reelin plays a central role in mammalian corticogenesis by regulating neuronal migration, adhesion, and final laminar positioning, constitutive Reelin loss-of-function models do not resolve its precise spatiotemporal requirements during telencephalic development. Furthermore, whether Reelin exerts evolutionarily conserved functions in vertebrate brains lacking a laminated cortex remains poorly understood.

To address these questions, we combined conditional genetics in the mouse with gene expression analysis in a zebrafish model.

First, we took advantage of conditional knock-out mouse models to analyze the effect of Reelin loss-of-function in distinct telencephalic domains and at specific developmental time points using Cre-lox strategies. Interestingly, Reelin deletion restricted to the dorsal telencephalon from embryonic day (E) 10.5 caused only mild alterations in cortical layer organization. In contrast, a broader deletion throughout the telencephalon starting as early as E9.5 markedly reduced Reelin expression and resulted in severe cortical disorganization, including the inversion of the laminar arrangement.

To investigate Reelin's role in a system in which the dorsal pallium is not laminated, we examined its expression in the teleost zebrafish using the Tg(*reln:Gal4;UAS:RFP*) line. During larval stages, Reelin-positive cells were detected in differentiated neuronal populations of the dorsal pallium and in defined subpallial territories, supporting the hypothesis that Reelin may contribute to neuronal positioning even in a non-laminated telencephalic context.

Together, these findings indicate that Reelin’s function in mammals is strongly dependent on its spatiotemporal expression context. Moreover, they point to a more ancient, evolutionarily conserved role for Reelin in vertebrate pallial development, which is the focus of our ongoing investigations.

Poster 9

From development to regeneration: Single-cell multiome analysis of neural progenitors in the salamander pallium

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The brain is an extremely complex organ that governs body functions and cognition, and contains a large variety of neuronal cell types. In mammals, neurogenesis is restricted mostly to embryonic stages, with the exception of a few brain regions retaining neural stem cells in the adult. In contrast, fish and salamanders have a widespread capacity to generate new neurons throughout their entire life cycle; building on this ability, these species can repair brain injuries by regenerating new neurons. However, why brain regeneration occurs in anamniotes but has been lost in mammals remains poorly understood. Understanding the mechanisms underlying adult neurogenesis and regeneration could open new perspectives for central nervous system regeneration in mammals.

Here, we hypothesize that differences of regenerative capacity across vertebrates trace back to evolutionary changes of neurogenesis and cell type diversity. We study a novel model organism, the Spanish ribbed newt *Pleurodeles waltl*, to characterize the evolution of cortical neurogenesis and regeneration at the cell type level. Recent research has shown that the dorsal telencephalon (the pallium) of *Pleurodeles* contains distinct neuron types that are localized to specific regions and specified by unique genetic programs. Here, using multiome single-cell profiling, we explore the diversity of neural progenitor cells along two distinct axes: developmental time (from pre-metamorphic larval stage to adulthood) and the regenerative process (during the early time course following pallial injury). This approach allows us to uncover dynamic changes in gene expression and chromatin accessibility in neural progenitor cells during regeneration, and to determine whether these changes recapitulate developmental neurogenesis programs.

Our results show similarities in gene expression and chromatin accessibility between neural progenitor cells during development and regeneration. We observe that regenerative progenitor cells decrease their depth of quiescence and resume their early proliferative capacity shortly after brain injury, a response that is critical for the regenerative outcome. We also identify progenitor clusters that are differentially enriched between larvae and adults, pointing to specific gene regulatory networks underlying brain development, homeostasis, and regeneration. The comparison of these molecular programs across species will be the entry point for elucidating the evolution of brain regeneration in vertebrates.

Poster 10

Next Generation Electrophysiology For Functional Characterization Of Human Neural Organoids And Assembloids

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Human-induced pluripotent stem cell (hiPSC)-derived 3D neural models (e.g. organoids, assembloids, etc.) are crucial tools for replicating human brain development and studying neurological disorders like Alzheimer's and Parkinson's disease. High-density microelectrode arrays (HD-MEAs) offer a label-free, non-invasive approach to real-time, high-resolution electrophysiological recordings from neural organoids, assembloids, and tissue explants.

We used the MaxOne and MaxTwo HD-MEA platforms, each featuring 26.400 electrodes per well, to record extracellular action potentials from various 3D neural models across different scales, ranging from cell population networks down to single-cell and subcellular levels. We showcased the flexible selection of recording electrodes, enhancing the data's reproducibility and statistical power. Key parameters, including firing rate, spike amplitude, and network burst profile, were extrapolated. The AxonTracking Assay was employed to trace action potential propagation along axonal branches and analyze conduction velocity, latency, and axonal morphology. This breakthrough assay enables high-resolution analysis of disease models targeting axon initial segment, development, and conduction.

The here presented HD-MEA platforms' capability for targeted electrode selection improves data consistency and enables more comprehensive statistical insights. Furthermore, automated data visualization and metric extraction make these systems a robust and user-friendly choice for in-vitro disease modeling and drug testing in both acute and longitudinal studies.

Poster 11

Columnar–laminar organization in the avian pallium reveals limits in current understanding of cortical evolution.

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In mammals and birds, the pallial telencephalon integrates sensory inputs in a manner that sustains behavioral plasticity. In mammals, this function is implemented by a six-layered neocortex with stereotyped connectivity, including radial recurrent circuits, horizontal layer-specific interareal projections, topographic innervation from thalamic sensory nuclei onto layer 4 and sparse inputs from thalamic matrix onto layers 2/3. In contrast, the avian pallium has long been considered non-laminar, a view recently challenged by evidence showing columnar-laminar organization in sensory areas.

Despite these similarities, the evolutionary relationship between avian and mammalian pallia remains controversial. Molecular studies suggest that much of the avian sensory pallium aligns with claustro-amygdalar territories rather than neocortex. However, accumulating structural and functional evidence calls for a more nuanced interpretation.

We examined the avian tectovisual pallium —previously shown as a three-layered structure (E-NI-M)— across four dimensions: intrinsic circuitry, efferent organization, thalamic afferents, and neurophysiology. Cellular reconstructions revealed distinct neuronal morphotypes in each layer, with axons spanning layers to form recurrent columnar circuits. Neurotracing showed efferents originating almost exclusively from NI neurons, targeting multiple pallial and one subpallial areas topographically, with all pallial recipients projecting back to NI — resembling the organization of mammalian supragranular layers.

We identified two thalamic afferents: a primary projection from nucleus rotundus to E, and a previously undescribed secondary projection from interstitial neurons to NI and associative areas, characterized by weak multisensory and descending pallial inputs, a consistent profile with matrix system in mammals. Electrophysiological recordings revealed visual responsiveness across all layers, while CSD analysis showed spatiotemporally recurrent synaptic activity. Correlated activity across aligned recording sites further supported functional columnar organization.

Together, these findings reveal limits in current explanations of pallial evolution by challenging the notion that molecular divergences preclude homology between avian and mammalian pallia. The degree of structural and functional resemblance documented here is too specific to be dismissed, and if this reflects convergence, it demands a mechanistic account of how independently evolving brain regions reach this level of equivalence. The question is no longer simply whether these structures are homologous, but what biological processes —conserved, convergent, or mixed— could produce such equivalent solutions.

Poster 12

Tools to decipher the developmental changes that diversified the thalamus of amniotes.

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The thalamus is a central brain structure that relays and integrates sensory and higher-order information across vertebrates. Understanding how its cellular diversity, connectivity, and developmental programs have evolved remains a major challenge in neuroscience. Here, we investigate the evolutionary origins of thalamic organization by combining developmental, transcriptomic, and connectivity analyses across amniotes, focusing on birds and reptiles, and integrating comparisons with mammals.

Using EdU-based birthdating approaches, we identified conserved neurogenic gradients underlying thalamic development. In both gecko and chicken, thalamic nuclei follow a common temporal sequence, with lateral and ventral regions generated earlier than medial domains, and the dorsomedial nucleus consistently arising last. These findings support the existence of developmental chronotopy, a shared rule across species that links temporal birthdating with anatomical location, while also revealing previously undescribed intra-nuclear gradients that may contribute to functional diversification.

To explore the relationship between development and connectivity, we performed retrograde axonal tracing experiments. Our current data suggest a correlation between the timing of neuronal generation and their connectivity patterns, indicating that early- and late-born populations may establish distinct circuit architectures. This relationship is supported by observations in gecko; however, in chicken, additional tracing experiments are required to confirm whether similar principles apply.

At the molecular level, we generated large-scale single-nucleus RNA sequencing datasets from embryonic thalamus in both species. These data indicate a conserved molecular profile across major neuronal populations between species.

However, using transcriptomic comparison and axonal tracing, we have identified lineage-specific adaptations, such as the presence of unique neuronal types in the mammalian anterior complex, suggesting evolutionary novelties. By integrating developmental, molecular, and connectivity data, we will uncover how conserved neurogenic processes shape thalamic structure while identifying modifications that drive species-specific complexity.

Poster 13

Laminated structure in the goby telencephalon: A new model to study evolution of cortical gyrification

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The development of the telencephalon in ray-finned fish differs from that of all other vertebrates in that it is formed through the process of eversion, resulting in two solid hemispheres separated by a common ventricle. This developmental process is believed to be responsible for the formation of a non-laminar telencephalon. Accordingly, ray-finned fish do not have a layered cortex and do not exhibit gyrification. Their telencephalon can be divided into an area dorsalis (D) and an area ventralis (V), which are considered equivalent to the pallium and subpallium. The telencephalic areas are organised in nuclei, named according to their location. Hence, layered structures are usually not found. The suborder of gobies (Gobioidei) is an exception here: A novel multilayered structure can be identified in the posterior region of the dorsal area. Visually, this area can be compared to the mammalian hippocampus. It consists of up to four regions surrounded by rows of cells. Using Nissl staining, approximately 90 goby species from six families were examined and compared with around 500 ray-finned fish species. This multilayered structure could only be detected in four goby families, which in turn show histological differences. All species examined in these families displayed this structure regardless of their lifestyle. However, this area is not present in the basal goby families. The multilayered cytoarchitecture of this structure is unique among ray finned fish to date. Its function and origin has not yet been investigated in depth.

We want to use this exceptional layered telencephalic structure as a model to explore molecular mechanisms underlying the evolution of cortical gyrification. It is planned to compare mRNA expression patterns at early developmental stages of gyrified versus non gyrified goby telencephalon. By using this approach, we hope to identify specific genes and/or gene expression patterns that underlie the surprising evolutionary emergence of telencephalic gyrification in this suborder of ray finned fish. This approach will be flanked immunohistochemistry and tracing techniques for the characterization of cell types in the gyrified structure.

Poster 14

Sauropsid embryonic brains lack the mammalian temporal transcriptional program

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Vertebrate brain neurogenesis is classically understood as being governed by spatially restricted genetic programs shaped by conserved morphogenetic gradients. More recently, studies in mammals have described a global temporal transcriptional code that sequences neuronal production, defining early, intermediate, and late progenitor and neuronal cells. However, the evolutionary conservation of this temporal program outside mammals remains unclear. Here, we investigated whether the mammalian temporal neurogenic code is conserved in sauropsids. We analyzed neurogenic regions of the embryonic chick telencephalon using *In Situ Sequencing* (ISS), enabling the simultaneous spatial and quantitative assessment of genes associated with early (e.g., *Onecut*, *Hmga1/2*), intermediate (e.g., *Pou2f2*, *Zfhx3*), and late (e.g., *Nfia/b*, *Tcf4*) neurogenic programs. We examined multiple developmental stages spanning early, mid, and late neurogenic windows. Our results show that sauropsid transcriptional profiles do not recapitulate the temporal progression described in mammals. Specifically, we do not observe a conserved sequential activation nor a clear segregation of molecular signatures corresponding to early, intermediate, or late neurogenic states. These findings suggest that the global temporal transcriptional program identified in mammals may not be conserved in sauropsids. Together, our data support the hypothesis that the mammalian temporal transcription code represents a lineage specific evolutionary innovation rather than a universal feature of amniote neurogenesis. This study highlights the power of spatial transcriptomics for addressing fundamental questions in evolutionary developmental neurobiology.

Poster 15

Formation, evolution and functional significance of cortical folds

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One of the most prominent features of the mammalian cerebral cortex is the presence of folds, namely gyri and sulci. Although cortical folds are thought to be important for higher brain functions, the developmental and evolutionary mechanisms of cortical folding as well as biological significance of cortical folds remain incompletely understood. Because mice lack cortical folds, we have been using ferrets, which possess a gyrencephalic cerebral cortex, as an experimental model. To investigate the genetic mechanisms in ferrets, we established genetic manipulation techniques for ferrets and demonstrated that fibroblast growth factor signaling and Sonic hedgehog signaling are important for cortical folding. We also showed that coordination between neurons and astrocytes is crucial for the formation of fully developed cortical folds.

We next explored the biological significance of cortical folds. Recent studies have shown that glymphatic circulation plays a crucial role in clearing metabolic waste, including amyloid beta, from the brain. We examined glymphatic circulation in ferrets and found cerebrospinal fluid (CSF) influx patterns that differ from those reported using mice. Specifically, CSF influx from the brain surface was markedly enhanced at sulci, and this sulcus-dominant CSF influx was mediated by aquaporin-4-positive astrocytes. These results indicates that sulci play important roles in enhancing glymphatic circulation efficiency in enlarged cerebrum. Our techniques in ferrets provide valuable tools for investigating the developmental and evolutionary mechanisms, as well as the biological significance, of the mammalian cerebral cortex.

Poster 16

Deep Learning-Based 3D Segmentation of Fetal Brain from Histology Across the Second Trimester

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Three-dimensional, region-wise morphometric characterisation of the developing human brain during early gestation remains limited, particularly in resolution and region parcellation. In histology, this is constrained by the extensive manual effort required to reconstruct serial histological sections and delineate regions across the entire brain. Our previous work, DHARANI, provides high-resolution three-dimensional histology volumes with detailed two-dimensional annotations on selected sections of early second trimester fetal brains (14–24 gestational weeks).

We present a U-Net–based deep learning approach that generates three-dimensional segmentations by extending annotations from a subset of histological sections to the full volume at 60 μm resolution. The anatomical ontology was consolidated from ~500 finely parcellated regions into 50 coarser regions to improve model robustness. Trained on the available annotations, the model produces spatially consistent region labels throughout the volume, resulting in whole-brain segmentation.

The reconstructed segmentations enable spatiotemporal, region-wise quantitative assessment of surface area, thickness and volume across development. Preliminary results indicate that structural maturation varies across brain regions, with distinct developmental patterns during this period. This work enables high-resolution quantification of early fetal brain development and supports the construction of normative morphometric references for the early second trimester.

Poster 17

Inducing mammalian cortex-like folding in chick brains

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Cerebral cortex folding emerges from the interplay between tissue growth and cytoarchitectural processes during embryonic and early postnatal development in mammals. Heterogeneous proliferation of neural progenitors establishes a protomap of alternating regions with high and low neurogenesis, followed by coordinated neuronal migration, collectively guiding future fold locations. While this cellular arrangement is considered a key driver of gyrification, limited access to mammalian embryos has hindered mechanistic studies of living tissue dynamics—such as growth anisotropy, cortical thickness, stiffness, and developmental timing—that underlie fold initiation. To investigate how these factors contribute to folding, we used the chick embryo, a tractable non-mammalian model that normally lacks cortical folds. By injecting Fibroblast Growth Factor 2 (FGF2), previously shown to induce folds in the mouse cerebral cortex, we tested whether this is sufficient to induce folding and, if so, use this paradigm to unravel the underlying mechanisms. Embryos injected with FGF2 at early stages displayed reproducible folds in the optic tectum (OT) by embryonic day 12, but not in the dorsal telencephalon (homolog to mammalian cerebral cortex). Remarkably, folds in OT appeared at conserved locations despite global application of FGF, suggesting intrinsic cytoarchitectural and geometric constraints. We are currently examining effects of FGF on the spatiotemporal patterns of cell-cycle progression, differentiation, and neuronal migration to elucidate the mechanisms underlying induced folding. Our work establishes the chick optic tectum as a tractable model for studying cellular and tissue-level events and mechanisms driving brain folding.

Poster 18

Evolution of human cortical development via transposable element exaptation into novel cis-regulatory elements

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The mammalian neocortex arose through coordinated innovations in excitatory and inhibitory neuron production, yet the genetic mechanisms enabling their co-evolution while maintaining excitation/inhibition (E/I) balance remain unknown. Here, I present evidence that transposable elements (TEs) provided a shared cis-regulatory substrate for the coordinated diversification of cortical cell types across vertebrate evolution. Analysis of predicted enhancers active in the developing human telencephalon reveals that three evolutionarily ancient TE subfamilies, LFSINE_vert, AmnSINE1, and MamRep434, are enriched within both excitatory and inhibitory neuron regulatory elements and harbor homeobox transcription factor motifs recognized by region-specific but motif-convergent factors including LHX2 (pallial, excitatory) and DLX1/2/5 (subpallial, inhibitory).

I hypothesize that recurrent TE insertions in the ancestors of tetrapods, amniotes, and mammals expanded the binding repertoire of these conserved homeobox factors, facilitating co-evolution of excitatory and inhibitory transcriptional programs. To test this, I am pursuing three complementary approaches: (1) comparative genomic and transgenic reporter analyses in mice to characterize the evolutionary conservation and in vivo activity of TE-derived cortical enhancers; (2) ChIP-seq profiling of LHX2 binding across mouse corticogenesis to map excitatory neuron TE occupancy and compare it with published DLX binding data from the subpallium; and (3) CRISPR interference-mediated silencing of TE-derived enhancers in excitatory and inhibitory neuron lineages to assess their functional impact on cortical neurogenesis and laminar organization. This work reveals a previously unappreciated mechanism by which selfish genetic elements were co-opted to coordinate cell-type diversification in the evolving cerebral cortex, with implications for understanding the genomic basis of E/I balance and associated cognitive disorders.

Poster 19

A functional single-cell atlas of Neanderthal variants in ancestralized human cerebral organoids

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Sequencing of the Neanderthal and Denisovan genomes has revealed fixed, protein-altering genetic changes that distinguish modern humans from other hominins. However, the effects of the majority of these SNPs in human neurodevelopment have not been investigated. We developed a prime-editing screening platform to introduce ancestral variants in human iPSC-derived cerebral organoids with transcriptome-wide readout. We tested 6000 pegRNAs across 80 targets to identify those that install ancestral, protein-altering SNPs, with some achieving >90% editing in human stem cells. To enhance detection of phenotypes emerging from homozygous editing, we established BRIDGE-seq (Barcode-Resolved Integration of Defined Genotypes to Expression in scRNA-seq), in which targeted scDNA-seq profiling editing outcomes is linked to transcriptomic effects via unique bridging barcode captured in both modalities. Using BRIDGE-seq, we profiled >120k high quality cells from D120 mosaic anscetralized organoids, with precise editing outcomes and zygosity for the 33 targets annotated in every cell. We identify cell-type-specific effects constrained to excitatory or inhibitory neuronal lineages for several Neanderthal variants, with some mutations resulting in layer-specific compositional effects. We find that subsets of ancestral variants convergently perturb gene networks involved in progenitor proliferation dynamics and cell fate decisions in a dose-dependent manner. BRIDGE-seq improves sensitivity by linking edit outcome to single-cell expression states in mosaic organoid screens, revealing changes human-specific genetic architecture resulting from single amino acid substitutions.

Poster 20

Dual Evolutionary Origins of Mammalian Subplate Neurons

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Subplate neurons (SpNs) are among the earliest-generated cortical neurons and are essential for the establishment of thalamocortical circuits in the mammalian neocortex. Despite their central role, their evolutionary origin has remained unresolved. Here, we performed comparative single-cell RNA sequencing and spatial transcriptomics across amniotes (mouse, chick, and turtle), revealing two distinct developmental and evolutionary origins of SpNs.

We identify (i) atypical SpNs (aSpNs), an Nr4a2-negative population conserved across amniotes, and (ii) mammalian-specific SpNs (mSpNs), an Nr4a2-positive population that is markedly expanded in mammals. aSpNs originate from the medial pallium and migrate tangentially into the subplate, representing an ancestral neuronal program. In contrast, mSpNs arise from early-born neurons in the cortical ventricular zone and correspond to a repurposed version of an ancestral input-neuron program observed in non-mammalian pallia.

Cross-species analyses show that early-born pallial neurons in birds and reptiles primarily differentiate into thalamic input neurons, characterized by Ctgf and Eag2 expression. In mammals, however, this ancestral program is repurposed: rather than forming permanent input neurons, early-born neurons instead transiently adopt a subplate identity.

We further demonstrate that this fate switch is controlled by the transcription factor Zbtb18. Although Zbtb18 is broadly expressed in the pallium, it is selectively repressed in mSpNs at a defined developmental stage, coinciding with the invasion of thalamocortical axons. This repression releases Nr4a2 from inhibition and promotes subplate neuron specification. Consistent with this model, disruption of thalamic input attenuates Zbtb18 repression, supporting an activity-dependent mechanism.

Together, these findings lead us to propose a dual-origin model in which the mammalian subplate arises from two sources: an ancestral medial pallium–derived lineage and a repurposed input-neuron program. This evolutionary innovation introduces an input-dependent layer that helps organize thalamocortical circuit assembly and may have enabled the expansion and increased complexity of the mammalian neocortex.

Poster 21

Developmental divergences in the evolution of the tectal mesencephalon of amniotes

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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.

Poster 22

Organization and Molecular Progression of Neural Progenitor Cells in the Developing Human Dentate Gyrus

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The complexity of human brain cytoarchitecture is established by neural progenitor cells (NPCs), foundational cellular populations that give rise to neurons and glia during development. The dentate gyrus (DG), key structure in learning and memory, maintains a tightly organized NPC population into adulthood in different mammalian species, retaining neurogenic potential beyond the developmental period. However, in the human DG the emergence, organization and persistence of NPCs remain incompletely characterized. NPCs have been reported in varying numbers in the adult and aged period, reflecting differences in identification criteria and highlighting the need for a unified framework across the full developmental continuum. In this study we provide a spatial and molecular map of the developmental trajectory of NPCs in the human DG, combining multimodal transcriptomic analysis within the neuroanatomical context. At mid-gestation, we observed changes in the structural and cellular arrangement in the hippocampus, coinciding with the emergence of an NPC multicellular layer within the DG, herein named the granular-hilar progenitor zone (GHPZ). Neurogenic transcriptomic signatures in the GHPZ were diminished by early infancy. This coincided with a reduction in NPC number and their progressive shift toward an astrocytic program. By childhood, the GHPZ was dissolved with only sparse radial NPCs remaining in the DG. Lastly, we validated WNT signaling pathway-associated genes as
NPC identity markers in the developing human DG, observing a decline in their expression after infancy. Our study defines the progression of NPCs from gestation to the postnatal period, showing a steep decline in the neurogenic potential by childhood, and sets the blueprint for NPC identification in the human DG.

Poster 23

Characterization, topography, and possible modulation of immature neurons in the mammalian cortical layer II

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Cortical immature neurons (cINs) are prenatally generated cells in arrested maturation, that can "awake" at different life stages to integrate into circuits of the cortical layer II. These cells are restricted to the piriform cortex in rodents and can be detected by immunocytochemistry since they express markers of undifferentiated neurons (e.g., the cytoskeletal protein doublecortin; DCX). We previously quantified the cINs in widely different mammals (from mouse to chimpanzee; young adult stage) showing that they are very abundant in gyrencephalic species, also extending into their neocortical mantle.

Here, the same approach (DCX immunocytochemical detection following cross-species neuroanatomical alignment) was used to extend the cIN quantitative analysis to young adult (n=4 entire brain hemispheres/species) dogs (Canis lupus, 1 year), horses (Equus caballus, 3–7 years) and macaques (Rhesus monkey, 7–10 years). The results obtained place cIN density of these species among the gyrencephalic mammals and confirm the covariance with both brain size and neocortical expansion.

Then, we studied cIN density across 16 neuroanatomically defined cortical regions in another group of macaques (n=11; 23 years). We found rather similar cIN density in the entire cortical mantle, with higher values in medial and inferior temporal cortex, as previously reported in the human temporal lobe, thus supporting the value of macaque as a good translational model for cIN study. We also investigated potential changes in cIN densities by comparing mother-reared Vs nursery-reared macaques in the 23-year-old group, without finding any statistically significant difference.

By contrast, ongoing work considering three groups of sheep exposed to different environmental conditions (enriched, isolated, and control; 9 months; 5 animals/group) for 8 weeks, showed a reduction in cIN density in both experimental settings compared to controls (suggesting an experience-dependent increased neuronal maturation), with statically significant decrease in the neocortex.

Overall, these findings support our hypothesis that cINs represent a cross-cutting feature of the cerebral cortex layer II (a cortico-cortical associative layer), not linked to functional specializations. Furthermore, we show that their maturation can be influenced by detours from routine life, suggesting that lifestyle can induce structural plasticity in the neocortical upper layers of gyrencephalic species.

Poster 24

Transcriptome-Resolved Precision Neuropathology for Malformations of Cortical Development

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The human cerebral cortex is assembled through spatially coordinated developmental programs that specify neuronal subtype identity and laminar positioning. Disruption of these programs produces malformations of cortical development (MCD), major causes of intellectual disability and drug-resistant epilepsy. Yet neuropathology still relies heavily on morphology and limited markers, limiting our ability to connect genotype to cellular mechanisms and intervention-relevant timing. We address this gap by applying spatial single-cell transcriptomics to map MCD pathogenesis through transcriptomically defined neuronal subtypes, anchored to our recently developed human developmental reference (Qian et al., Nature, 2025). Applying this framework to postmortem and surgical MCD specimens reveals subtype-resolved lamination phenotypes that are not captured by traditional methods. Across etiologies, pathology can be quantified as coordinated shifts in subtype composition and spatial positioning, enabling cross-disorder comparisons that highlight convergent vulnerabilities and nominate mechanistic hypotheses.

In LIS1-associated lissencephaly, spatial transcriptomics overturn a simple global layer inversion model by resolving subtype-selective mis-localization: deep-layer programs and subsets of Layer 4 populations preserve inside-out ordering, while specific upper-layer subtypes shift to deeper positions. In parallel, inhibitory populations show selective vulnerability, including depletion of PV and SST interneuron subtypes, consistent with circuit-level imbalance and temporally restricted migration disruption.

In focal cortical dysplasia (FCD) resections, we observe an analogous principle of subtype-selective dyslamination coupled to disease-associated state changes. Laminar excitatory neuron organizations remain detectable but show marked broadening of depth distributions relative to adjacent cortex. Lesion-enriched excitatory populations are distributed across gray and white matter with mixed transcriptional signatures and align with dysmorphic neuron-associated features, while glial state remodeling tracks with dyslamination and transitions across the lesion boundary. Spatial transcriptomics also identified lesion-specific neuronal populations in FCD that express pS6, consistent with mTOR hyperactivation, and are entirely absent from control cortex. These cells express neuronal genes alongside aberrant low-level expression of glia-associated genes, suggesting unstable or confused cellular identity.

This integrated developmental and disease framework establishes transcriptome-resolved precision neuropathology as a scalable strategy to define vulnerable cell populations and developmentally restricted windows for intervention in neurodevelopmental disorders.

Poster 25

When time shapes structure: cell cycle length during neurogenesis in amniotes

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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.

Poster 26

Why can we speak? The role of Foxp2 in learning and language

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Foxp2 was the first gene directly linked to the uniquely human ability of vocal learning in the primate lineage; a heterozygous mutation in the gene renders humans unable to speak. Additionally, it was shown that two amino acid substitutions occurred on Foxp2 during human evolution, which is more than expected given its conservation, further stressing its influence on human language.
Introducing the humanized version of Foxp2 in mice facilitates better performance in learning-based assays and altered transcriptomes in the striatum compared to wild-type animals.

To better understand the molecular effects of Foxp2 on learning, we performed a large learning screen of >100 mice (humanized Foxp2 vs. WT vs. Foxp2 KO) at different stages of learning using RNA-seq, finding a direct molecular signal of altered learning in the humanized Foxp2 mice, containing genes associated with synapse remodeling as well as immediate early genes. Immediate early genes bridge neuronal activation to adjust synaptic strengths by ultimately altering neuronal transcription or protein states. This enables experience-dependent neural circuit remodeling necessary for learning. Analyzing the spatial expression patterns of immediate early genes in the temporal context of learning allows us to understand how these molecular changes in the brains of humanized Foxp2 mice lead to improved learning performance. To this end, we developed a cost-efficient end-to-end spatial transcriptomics workflow, with an integrated automated image analysis with single-cell and single-transcript resolution. This pipeline enables us to track the expression of the immediate-early genes across the brain samples from our previous learning screen. This could yield crucial insights into the complexity of learning and the role of Fopx2 in vocal learning.

Poster 27

oRG-like progenitor cells in the embryonic mouse dentate gyrus: implications for the evolutionary origin of cortical folding

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Outer radial glia (oRG) cells are abventricularly dividing progenitors that maintain a basal process and are widely considered key contributors to neocortical gyrification. First characterised in the developing human and ferret neocortex, oRG have since been identified in other gyrencephalic species and, in small numbers, in the lissencephalic mouse neocortex, where they are enriched medially. Despite growing interest in oRG biology, all reports to date have been restricted to the neocortex.

The hippocampal dentate gyrus presents a compelling test case. A phylogenetically ancient cortical structure, the dentate gyrus is folded in virtually all mammals, including monotremes and marsupials, in stark contrast to the highly variable gyrification of the neocortex. Whether progenitors comparable to neocortical oRG contribute to this conserved fold has not been investigated, owing partly to the technical difficulty of targeting the developing dentate gyrus with gene delivery approaches.

We established a novel *in utero* electroporation protocol that achieves reliable gene transfer to the embryonic mouse dentate gyrus across multiple developmental stages. Using this method, we identified a previously undescribed progenitor population. These cells occupy abventricular positions, extend a basal process towards the hippocampal fissure, lack an apical attachment, and are proliferative. Time-lapse imaging revealed somal translocation-like behaviour preceding cell division. Immunohistochemical analysis demonstrated co-expression of Pax6 and Hopx, markers associated with neocortical oRG identity. We designate these cells dentate gyrus oRG-like progenitors. They exhibited a more undifferentiated molecular profile than oRG reported in the mouse neocortex, including the medial cortex where neocortical oRG are comparatively enriched.

Our findings demonstrate that progenitors with oRG characteristics are not confined to the neocortex but are present in a conserved archicortical fold, bridging evolutionary and developmental timescales: a progenitor programme active during embryonic neurogenesis may trace its origins to the base of mammalian cortical evolution. The dentate gyrus may thus retain an ancestral folding mechanism that has been lost from the neocortex of lissencephalic species.

Poster 28

Ablation of Abracl in the Nkx2-1 progeny affects MGE proliferation and the tangential migration of cortical interneurons.

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Abracl is a non-typical winged-helix protein exclusively expressed in the forebrain during mammalian embryogenesis; it is detected in proliferating and postmitotic progenitors, migrating interneurons, and transiently in fiber tracts, suggesting a role in cell proliferation and migration. To explore its role during embryonic brain development, we generated a conditional Abracl knock-out (KO) mouse model using the Cre-loxP system. LoxP sites were introduced flanking exon 3 of *Abracl*, which encodes most of the protein sequence, including the stop codon. These *Abracl*$^{flox/flox}$ mice were crossed with *Nkx2-1Tg(Cre);R26loxP-STOP-loxP-YFP* mice, allowing the conditional Abracl deletion in *Nkx2-1* progeny. Analysis of the brain of KO embryos between E11.5 and E13.5, revealed significant aberrations in the generation and the programmed cell death of MGE progenitors. Moreover, quantitative analysis showed that the Lhx6- and the *Sst*-positive migrating cortical interneurons per square millimeter of pallial tissue, was approximately 9 fold lower in KO than in WT embryos. Interestingly, in heterozygous embryos, the number of Lhx6-positive cells in the pallium was reduced by approximately 2.5 fold. Despite this disruption, the expression patterns of the subpallial markers *Nkx2-1*, *Dlx2*, *Dlx5*, and *Gad1* showed no significant aberration. These findings suggest that Abracl has a role in the regulation of cell proliferation within the MGE and/or tangential migration of cortical interneurons, without affecting however subpallial patterning.

Poster 29

Cell to volume characterization of the fetal Hippocampal Formation reveals early modular architecture of the presubiculum and pronounced volumetric expansion in the second trimester

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The human hippocampal formation (HCF) undergoes extensive geometrical and cellular reorganization during development, yet the spatiotemporal dynamics underlying its divergence from rodent models remain poorly understood. In this study, we use an integrated approach of high-resolution 3D-reconstructed histology, registered to the MRI, to characterise and quantify cell-to-volumetric changes in the HCF and its subregions. The morphometric analysis is based on a high sampling of 100-400 annotated histological sections per specimen from 14-24 GW. The developing HCF shows coordinated volumetric expansion, folding, and the emergence of immunohistochemically defined subregional boundaries, bridging the gaps left by 2D histology and MRI. Our integrated approach provides a systematic quantification of subregional volumetric surface-area expansion. It shows that the entire HCF undergoes angular rotations of 18°, 5°, and 4° in the anterior-posterior, medio-lateral, and superior-inferior planes, respectively. The subregional quantification showed maximum volume expansion of the presubiculum, nearly 8- fold, during the second trimester. The pronounced geometric expansion of the presubiculum is particularly striking, given its pivotal role in spatial navigation circuitry. Cellular architecture of the cell islands in the presubiculum revealed complementary PCP4 and Calbindin expression, providing the first evidence of the modular organization of head-direction cells as early as 21GW. Notably, this complementary expression differs from that in adult rodent models, where Calbindin negative patches lack PCP4 expression, highlighting cross-species differences. Although PCP4 expression in the neuropil is commonly used to delineate CA2 fields in the adult human hippocampus, no PCP4 expression was detected in this region up to 24 GW. CA fields showed layer-specific calbindin expressions. Overall, our data provides a cell-to-volume framework for the developing human HCF.

Poster 30

Wnt-dependent switch to radial glial fiber-independent bipolar migration in the avian pallium

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The evolutionary divergence between the laminated mammalian neocortex and the nuclear organization of the avian pallium remains a central question in neuroscience. In mammals, excitatory neurons migrate along radial glial fibers (RGFs) via locomotion, which is essential for cortical layer formation. In contrast, neurons in reptiles have been thought to rely mainly on slow, non-directional multipolar migration, but remains unknown in the avian pallium.

Here, we identify a novel migration mode in the developing chick dorsal pallium: a bipolar, highly directional mode that enables rapid long-distance neuronal movement. Using long-term live imaging and lineage tracing, we show that chick neurons undergo a developmental transition from an initial multipolar phase to this bipolar migration mode.

This transition is tightly regulated by downregulation of canonical Wnt signaling, paralleling the multipolar-to-locomotion switch in mammals. However, unlike mammalian neurons that migrate along with RGFs, chick bipolar neurons detach from their radial scaffold upon entering the superficial pallium. Quantitative analyses reveal that their leading processes are oriented largely independently of the local RGF architecture.
Together, our findings suggest that birds and mammals share a conserved Wnt-dependent molecular program for fast neuronal migration, but deploy distinct cellular strategies—RGF-dependent versus RGF-independent—to generate divergent brain architectures.