Programme Overview (22-24 May 2024)

The Spanish Society of Neuroscience (SENC) announces the celebration of the 12th Cajal Conference. The aim of this series of Conferences is to bring together specialists in the Neuroscience area to present their recent data and discuss with international leaders in the field on common interests in an open, informal atmosphere.

The field of epigenetics has introduced a novel branch of study that aims to elucidate the mechanisms through which the environment can modify individual genomes shaping new biological functions regardless of classic genetic inheritance. The environment, life style and social interactions modulate changes in gene expression that together with inherited risk variants confers susceptibility for mental conditions.

The sexual skewing observed across all psychiatric disorders suggests that males and female respond in different ways to genetic and epigenetic risk factors for mental disorders. Sex-specific developmental mechanisms could lead to differences in the susceptibility or protection to mental disorders amongst male and female.

The proposal of this conference dwells on the discussion of novel perspectives of epigenetics related to mental conditions and how sex factors may influence the differential risk for psychiatric conditions observed in males and females. We will focus on the implementation of human organoid models to untangle the contribution of epi-modulators and sex-related factors to behaviours and mental conditions.

Local Organizing team:

- **Dr. Esther Serrano Saiz** – CBMSO (Organ and Tissue Homeostasis)
- **Dr. Claudio Toma** – CBMSO (Physiological and pathological processes)
- **Dr. Leonardo Beccari** – CBMSO (Organ and Tissue Homeostasis)

Schedule:

	Wednesday 22	Thursday 23	Friday 24
7:30 – 9:00		Breakfast	Breakfast
9:30 –11:00		Oral Presentations (3 speakers)	Recreational Activity (Kayaking, Camino de Ronda, Platja d'Aro)
11:00 – 12:00	Registration Opens	**Nirao Shah** (Stanford University, USA)	
12:00 – 13:30	Welcome	Free time	
13:30 – 15:00	Lunch	Lunch	Lunch
15:30 – 16:30	**Ángel Barco** (Instituto Neurociencias, Spain)	**Margaret McCarthy** (University of Maryland, USA)	**Thomas Bourgeron** (Institut Pasteur, France)
16:30 – 18:00	Oral Presentations (3 speakers)	Oral Presentations (3 speakers)	Oral Presentations (3 speakers)
18:00 – 18:30	Coffee Break	Coffee Break	Coffee Break
18:30 – 19:30	**Jose González Martínez** (MRC LBM, UK)	**Marian Martínez** (IBMB, Spain)	**András Lakatos** (University of Cambridge, UK)
19:30 – 20:30	Poster session	Poster session	
21:00 –	Dinner	Dinner	Dinner

Invited Speakers:

- **Ángel Barco:** *Epigenetic mechanisms in neuronal plasticity and neurological disorders*
- **Margaret McCarthy:** *Sex Differences in the Developing Brain: Intersection of the Endocrine, Immune and Nervous System*
- **András Lakatos:** *Early chromatin accessibility and transcriptomic changes revealed by human organoid models of neurodegeneration*
- **Marian Martínez:** *JMJD3 integrates lineal and tridimensional information upon TGFb signal during early neurogenesis*
- **Nirao Shah:** *Neuromodulatory control of innate social behaviors*
- **Thomas Bourgeron:** *The genetic architecture of autism: from medicine to neurodiversity*
- **José González Martínez:** *Investigating the interaction between autism risk genes and androgens during neurogenesis*

Selected talks from abstracts:

	Oliver Davis *(16.30-17.00): MLL2* has a methylation-independent role in tethering enhancer-promoter chromatin loops during pluripotent cell priming of neuroectoderm differentiation
Wednesday 22 16:30 – 18:00	**Ernest Palomer Vila** *(17.00-17.30):* Sirt2 represses synaptic Frizzled receptors at early stages of Alzheimer's Disease
	Rafael Casado-Navarro *(17.30-18.00): Dmrt5* function in the mouse midbrain might explain susceptibility to attention-deficit/hyperactivity disorder in the male population
	David Sánchez Pizarro *(9.30-10.00):* Deficiency of the nutrient sensor Cpt1c in SF1 neurons disrupts the Endocannabinoid system resulting in compromised satiety and fuel selection upon fat intake
Thursday 23 9:30 –11:00	**Marta Cosin Tomas** *(10.00-10.30):* Assessing the Link Between Placental DNA Methylation and Offspring Cognitive and Behavioral Outcomes
	Inés García Ortiz *(10.30-11.00):* RNA sequencing analyses in bipolar multiplex bipolar families point to novel genes and dysregulated pathways
	César Díaz García *(16.30-17.00):* Alterations of the endocanabinoid system and synaptic plasticity in an in vivo preclinical model of periodontitis and depression
Thursday 23 16:30 – 18:00	**Marina Mitjans Niubo** *(17.00-17.30):* Shared vulnerability and sex-dependent polygenic burden in psychotic disorders
	Miriam Martínez Jiménez *(17.30-18.00):* Study of alternative splicing events from RNA-seq in multiplex bipolar families
	Sara Cacciato Salcedo *(16.30-17.00):* Atypical predictive processing in the inferior colliculus of the valproic acid-induced rat model of Autism
Friday 24 16:30 – 18:00	**Pablo Mendez** *(17.00-17.30):* Impact of gonadal and genetic sex on hippocampal function
	Ana Bermejo Santos *(17.30-18.00):* Doublesex and mab-3 related transcription factor 2 (*Dmrt2*) regulates the development of cingulate cortex neurons.

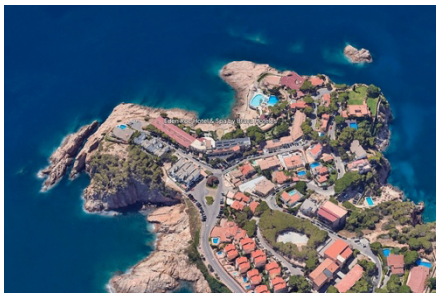
Travel grants:

The SENC offers **10 travel grants** for PhD students and Postdocs that will cover the registration to the meeting. Applicants must be SENC members (membership link https://www.senc.es/en/how-to-join-2/). To apply, upload your CV and an abstract during the registration process: https://amconferences.eventsair.com/senc-cajal-conference-2024/inscripcion/Site/Register .

Venue

Eden Roc Mediterranean Hotel and Spa

The summit will take place at the **Eden Roc Mediterranean Hotel and Spa**. located in the heart of the Costa Brava, at the seaside and surrounded by the Mediterranean Sea. The Hotel was built in the 70s on top of the cliff in the old baths of St Elm and has 140 rooms, most of them with terraces overlooking the sea. The idyllic old fishing village of Sant Feliu de Guíxols is at a distance of 800 m and still preserves an important historical heritage, including the Benedictine Monastery.



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Abstracts

Oral communications

MLL2 has a methylation-independent role in tethering enhancer-promoter chromatin loops during pluripotent cell priming of neuroectoderm differentiation.

Davis O, Steindel S, Pirvan L, Neumann K, Adhya D, Morf J, Agsu G, Wurmser A, Strawbridge SE, Zhang Z, Huntly B, Mohorianu I, Holcman D, Klenerman D, Samarajiwa SA, Anastassiadis K, Stewart AF, Basu S.

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Pluripotent cells (PCs) of the embryo give rise to all the differentiated cell types of an adult tissue. To do this, PCs undergo a co-ordinated program of differentiation, which involves progressive changes in the transcriptome. This involves a "priming" stage where the PCs undergo transcriptional and epigenetic changes that subsequently enable the cells to differentiate into other lineages. The mechanisms controlling these changes and PC differentiation are an active area of research.
Here, we study how control of 3D genome architecture influences transcription during mouse PC priming. In particular, we focus on the role of MLL2, a chromatin regulator with a canonical role of depositing histone H3 K4 trimethylation marks at bivalent promoters. Loss of MLL2 influences PC differentiation, with a loss of neuroectodermal cell fate. Interestingly, this phenotypic effect is time-dependent; loss in early naïve (unprimed) PCs leads to a neuroectodermal phenotype, but loss in primed PCs does not.
To explore the mechanism behind this time-dependent phenotype, we used conditional MLL2 knockout mouse PCs and performed a time-course of single cell RNA sequencing as naïve PCs transition into primed PCs. Our results show that loss of MLL2 does not cause a large-scale change in the transcriptome. Instead, we identify expression changes in specific lineage-specifying genes during the transition.
To extend our transcriptional findings, we next determined the effect of MLL2 perturbation on 3D genome architecture by using Micro-C of naïve and primed PCs. We identified that loss of MLL2 affected chromatin loop formation with a loss of enhancer-promoter and promoter loops, particularly at bivalent genes normally expressed during the differentiation of mouse PCs. This loss of chromatin loops was rescued by a mutant MLL2 protein with no methylating ability, showing a methylation-independent role for MLL2 in controlling 3D genome architecture during the priming of PCs. It suggests MLL2 has a role in tethering and forming enhancer-promoter loops in primed PCs. This is supported by single-molecule tracking of histone H2B showing that MLL2 restricts chromatin mobility as naïve PCs transition into primed PCs. Together, our data reveals a novel mechanism involving the control of 3D genome architecture for how MLL2 controls stem cell differentiation

Sirt2 represses synaptic Frizzled receptors at early stages of Alzheimer's Disease

Ernest Palomer[1,2], Núria Martin-Flores[1], Sarah Jolly[3], Patricia Pascual-Vargas[1], Stefano Benvegnù[3], Marina Podpolny[1], Kadi Vaher[1], Paul Whiting[3,4], Patricia C Salinas[1]

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Growing evidence supports a role for deficient Wnt signalling in synapse degeneration in Alzheimer´s disease (AD). First, the Wnt antagonist DKK1 is elevated in the AD brain and is required for amyloid-β-induced synapse loss. Second, LRP6 Wnt co-receptor is required for synapse integrity and three variants of this receptor are linked to late-onset AD. However, the expression and role of other Wnt signalling components remain poorly explored in AD. Among the different Wnt signalling components, Wnt receptors Frizzled1 (Fzd1), Fzd5, Fzd7 and Fzd9 are of particular interest due to their role in synapse formation and plasticity. Our analyses showed that *FZD1* and *FZD7* mRNA levels were reduced in the hippocampus of human preclinical AD (PAD) cases and in the $hAPP^{NLGF/NLGF}$ mouse model. This transcriptional downregulation was accompanied by reduced levels of the pro-transcriptional histone mark H4K16ac and a concomitant increase of its deacetylase Sirt2 at *Fzd1* and *Fzd7* promoters in AD. *In vitro* and *in vivo* inhibition of Sirt2 rescued *Fzd1* and *Fzd7* mRNA expression and H4K16ac levels at their promoters. In addition, we showed that Sirt2 recruitment to *Fzd1* and *Fzd7* promoters is dependent on FoxO1 activity in AD, thus acting as a co-repressor. Finally, we found reduced levels of inhibitory phosphorylation on Sirt2 in nuclear PAD samples and increased levels of the Sirt2 phosphatase PP2C, leading to hyperactive nuclear Sirt2 and favouring *Fzd1* and *Fzd7* repression in AD. Collectively, our findings define a novel role for nuclear hyperactivated Sirt2 in repressing *Fzd1* and *Fzd7* expression *via* H4K16ac deacetylation in AD. These results postulate Sirt2 inhibition as a therapeutic target for boosting Wnt signalling and ameliorate AD.

Dmrt5 **function in the mouse midbrain might explain susceptibility to attention-deficit/hyperactivity disorder in the male population**

Rafael Casado-Navarro[1], Javier Macho Rendón[2], Inés García-Ortiz[1], María Pilar Madrigal[3], Sonia Amorós[3], Jose Ignacio Gomez-Blanco[1], Juan Ramón Martínez-Morales[2], Sandra Jurado[3], Claudio Toma[1,4,5], and Esther Serrano-Saiz[1]

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The mammalian brain presents sex differences at molecular and structural levels. Gonadal hormones, genes and epigenetics contribute to the generation of these sex differences and might be crucial for the sex bias in mental disorders. Recently, members of the ancient family of *Dmrt* genes (*doublesex* and *mab-3* related transcription factors) have emerged as conserved regulators of sex-specific traits across metazoans, playing a key role in the sexual differentiation of the nervous system in invertebrates. However, it is unknown whether *Dmrt* genes regulate the sexual differentiation of the mammalian brain. In mice, *Dmrt5* is well characterized in the control of cortex development, and in humans, a mutation in *DMRTA2/DMRT5* leads to a recessive condition characterized by microcephaly and lissencephaly. We performed genetic analyses across 10 psychiatric phenotypes and found association between DMRTA2/DMRT5 and attention deficit/hyperactivity disorder (ADHD) (Gene-based P = 5.8E-4), a condition that shows higher prevalence among male population and evidence for alterations in cortex and midbrain. This study aims to unravel *Dmrt5* sex-specific functions in the mouse midbrain.
We show that *Dmrt5* null mutant mice (*Dmrt5-/-*) present patterning defects within the midbrain at early developmental stages, evidenced by the miss-expression of transcription factors such as *Barhl1, Engrailed1* or *Foxa1*. Furthermore, the midbrain of *Dmrt5-/-* mutants of both sexes shows fewer tyrosine hydroxylase positive (TH+) neurons, and the remaining TH+ neurons send defasciculated axons. Additionally, we compared the transcriptional profiles of males and females *Dmrt5-/-* mutants at late

developmental embryos, coinciding with embryonic testosterone release. Our analysis revealed that *Dmrt5* prevents differences in gene expression between males and females.
Interestingly, the human orthologous genes dysregulated in *Dmrt5* mutant males (N=166) were associated to ADHD in a gene-set analysis (P=0.004) using genome-wide association studies (GWAS) (38,691 ADHD cases; 186,843 controls). The mis-regulation of these gene networks might predispose for ADHD with a stronger prevalence in the male population. In conclusion, *Dmrt5* emerges as a crucial transcription factor required for the accurate development of the midbrain and might be necessary for achieving a transcriptome equivalence between male and female midbrains.

Deficiency of the nutrient sensor cpt1c in sf1 neurons disrupts the endocannabinoid system resulting in compromised satiety and fuel selection upon fat intake

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The SF1 neurons of the ventromedial hypothalamus (VMH) are pivotal in governing body weight and adiposity, particularly in response to a high-fat diet (HFD). Previous studies have shown that the activation of SF1 neurons induces satiety, increases energy expenditure, and promotes the preferential use of fats as energy substrate. Furthermore, SF1 neurons are necessary for recovering from insulin-induced hypoglycemia. Here we demonstrate the essential role of the nutritional sensor CPT1c in the activation of SF1 neurons by dietary fats. Mice deficient in CPT1C in SF1 neurons (SF1-CPT1c-KO) are unable to adjust their caloric intake during the initial exposure to a HFD. This is associated with an impaired metabolic transition in the liver, muscle, and adipose tissue, despite a normal response to a glucose or insulin challenge. During chronic HFD exposure, SF1-CPT1c-KO mice are more prone to obesity and glucose intolerance than controls. CPT1c deficiency in SF1 neurons also leads to alterations in hypothalamic endocannabinoid levels and their metabolism. Our findings posit CPT1C in SF1 neurons as a sensor for dietary fats, regulating satiety responses and nutrient partitioning likely through the modulation of the endocannabinoid system.

Assessing the Link Between Placental DNA Methylation and Offspring Cognitive and Behavioral Outcomes

Laia Diez, MSc.[1,2,3]; Ariadna Cislleros-Portet, MSc.[4];Nora Fernández-Jimenez, PhD.[4]; Mariana F. Fernández, PhD.[3,5]; Monica Guxens, PhD.[1,2,3,6]; Jordi Julvez, PhD.[1,2,3,7]; Sabrina Llop, PhD.[3,8]; Maria-Jose Lopez-Espinosa, PhD.[3,8,9]; Mikel Subiza, PhD.[3,10,11,12]; Manuel Lozano, PhD.[8,13]; Jesus Ibarluzea, PhD.[3,12,14]; Jordi Sunyer, PhD.[1,2,3]; Mariona Bustamante, PhD.[1,2,3]; Marta Cosin-Tomas, PhD.[1,2,3 #]

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Placenta plays a crucial role protecting the foetus from environmental harm and supports the development of its brain. In fact, compromised placental function could predispose an individual to neurodevelopmental disorders. Placental epigenetic modifications, including DNA methylation, could be considered a proxy of placental function and thus plausible mediators of the association between intrauterine environmental exposures and genetics, and childhood and adult mental health. Although neurodevelopmental disorders such as autism spectrum disorder have been investigated in relation to placenta DNA methylation, no studies have addressed the association between placenta DNA methylation and child's cognitive functions. Thus, our goal here was to investigate whether placental DNA methylation profile measured using the Illumina EPIC array is associated with three different cognitive domains (namely verbal score, perceptive performance score, and general cognitive score) assessed by the McCarthy Scales of Children's functions in childhood at age 4. To this end, we conducted epigenome-wide association analyses including data from 255 mother-child pairs within the INMA project and performed a follow-up functional analysis to help the interpretation of the findings. After multiple-testing correction, we found that methylation at 4 CpGs (cg1548200, cg02986379, cg00866476 and cg14113931) was significantly associated with the general cognitive score, and 2 distinct differentially methylated regions (DMRs) (including 27 CpGs) were significantly associated with each cognitive dimension. Interestingly, the genes annotated to these CpGs were involved in placenta, foetal, and brain development. These findings suggest that placental DNA methylation could be a mechanism contributing to the alteration of important pathways in the placenta that have a consequence on the offspring's brain development and cognitive function. Furthermore, we are currently conducting analyses on the association between placenta DNA methylation and neurodevelopmental outcomes at 18 months of age assessed with the Bayley Scales of infant development by a neuropsychologist in the BiSC cohort (Barcelona Life Study Cohort). Analyses stratifying by sex are also ongoing for both datasets.

RNA sequencing analyses in bipolar multiplex bipolar families point to novel genes and dysregulated pathways

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Bipolar disorder (BD) is characterized by fluctuations between mania and depression with a strong genetic component. The genetic architecture of BD comprises multiple loci, including common risk variants of small effect and rare risk variants of higher penetrance. Whole exome or genome sequencing in multiplex families have rapidly accelerated the identification of candidate genes for the disorder while RNA sequencing (RNAseq) studies in BD are still limited. We performed RNAseq analyses in a cohort of multiplex bipolar families aiming to identify candidate genes and pathways involved in the disorder. RNA-seq was performed on total mRNA extracted from lymphoblastoid cell lines of 16 BD and 15 unaffected individuals from eight bipolar multiplex families. We performed differential gene expression analysis (DGE) and weighted gene coexpression network analysis (WGCNA) using DESeq2 and WGCNA software, respectively. For both analyses we accounted for 'age', 'sex' and 'pedigree' as covariates. Differentially expressed genes (DEGs) and co-expressed modules were examined for enriched categories via gene set enrichment analysis (GSEA) and over-representation analysis (ORA), respectively. The DEGs and enriched
categories were validated using MAGMA and PoPS software, using as input file the GWAS summary statistics of BD (41,917BD cases; 371,549 controls). Validation of DEGs using RNAseq data derived from human brain will be an important next step, which is currently underway in an independent cohort from the PsychENCODE dataset (RNAseq of 73BD;259 controls). Sixty genes were found to be significant for differential expression between affected and unaffected relatives, the most significant being LINC01237. High correlation (r=0.83) of this set of DEGs was found between the lymphoblastoid cell lines and the average of gene expression across 13 brain tissues; 56/60 DEGs showed basal expression in brain (>0.1 TPM; https://gtexportal.org). Four DEGs, CEBPZ, GNB2, PPDPF and RC3H1, were pinpointed by both MAGMA and PoPS analyses for genetic association to BD. GSEA identified 500 significant enriched categories from 7 curated databases, and MAGMA gene-set analysis validated 68 of them. The top validated categories were related to signalling in the nervous system and ion transmembrane transport. WGCNA built 47 modules and 9 of them were significantly associated with BD. The ORA analysis found 281 enriched GO terms in 5 of the associated modules: 11 categories were validated via MAGMA gene-set analysis, "hypoplasia of corpus callosum" being the most significant category. We found long non-coding RNA, LINC01829 and LINC01237, in our DEGs, suggesting that this class of regulatory modulators may be key elements in the pathogenesis of BD. Our validated DEGs (CEBPZ, GNB2, PPDPF and RC3H1) are genes related to signal transduction, response to stimuli and inflammasome. Their function in the brain and previous genomic evidence make them valid candidate genes for BD. Enriched categories from GSEA and ORA after validation pointed to: i) signalling crucial for brain maturation and maintenance, including Wnt, hedgehog, and ROBO receptors pathways; ii) ion channel transport, which dysregulation may affect the action potential in neurons; iii) 'hypoplasia of corpus callosum', which has been reported to be altered in the largest imaging study in BD.

Alterations of the endocanabinoid system and synaptic plasticity in an in vivo preclinical model of periodontitis and depression

César Díaz-García[1,2], Javier Robledo-Montaña[1,2], María Martínez[3,4], Leire Virto[3,4], Mariano Sanz[3,4], Juan C. Leza[1,2], Borja García-Bueno[1,2], Elena Figuero[3,4#], David Martín-Hernández[1,2#]

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Periodontitis is an irreversible inflammatory condition caused by chronic accumulation of bacterial plaque on the teeth that affects the surrounding and supporting tissues. Depression is a highly prevalent mood disorder whose etiopathogenesis is still not fully understood, but one of the theories that tries to explain it is based on its inflammatory origin. Alterations of the endocannabinoid system and synaptic plasticity have been reported in inflammatory diseases, therefore, in order to study the role of inflammation on these phenomena, an in vivo preclinical model of periodontitis and depression (Perio+CMS) that combines both diseases has been developed. The results of the study suggest that in the combined model there is a global dysfunction of the endocannabinoid system, both at the level of the synthesis (NAPE-PLD, DAGL) and degradation (FAAH, MAGL) enzymes and the receptors (CB1, CB2), although an increase in the CB2 receptor is observed in microglia which could constitute a defense mechanism to be explored. Likewise, alterations have been found in the proteins involved in synaptic plasticity, specifically those involved in the BDNF signaling pathway. The combined model demonstrates the presence of changes in the endocannabinoid system and synaptic plasticity, showing that both processes could be interconnected and can be affected in a context of inflammatory disease.

Shared vulnerability and sex-dependent polygenic burden in psychotic disorders

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There is increasing evidence for shared genetic susceptibility between psychiatric disorders. The PGC cross-disorder group has provided molecular evidence for this common genetic architecture clustering

schizophrenia (SZ), bipolar disorder (BD) and major depressive disorder (MDD) in the mood and psychotic disorders group (Lee et al. 2021). However, although sex differences have been described in relation to clinical features and outcome in psychotic disorders (Riecher-Rössler et al. 2018), sex differences at the genetic level have been so far under-explored.
The aims of this study were: i) to evaluate the association of polygenic scores (PGSs) for SZ, BD and MDD with disorders of the psychotic spectrum in a sample of patients with broadly defined psychosis and ii) to test whether sex differences exist in those associations.
Our study included 1826 patients (33.2% females) with a range of psychotic disorders (DSM-IV criteria) and 1372 healthy controls (45.8% females) from the Spanish population (CIBERSAM cohort). Samples were genotyped with the Infinium PsychArray (Illumina) and imputation was conducted in the Michigan Imputation Server. PGSs were calculated using PRS-CS tool (Ge et al. 2019) and PLINK 1.9 (Chang et al. 2015) based on the following GWAS: SZ (Trubetskoy et al. 2022), BD (Mullins et al. 2021) and MDD (Wray et al. 2018).
Logistic regression models were used to study the association between psychotic disorder diagnosis and the PGSs (sex, centre and 10 PCs as covariates). To quantify the increased risk, the sample was divided into quartiles based on SZ-PGS, BD-PGS and MD-PGS and the odds ratios (ORs) were calculated for affected status in each quartile using the first quartile as reference. Secondary analyses were run stratified by sex. We found an association between all PGSs and broadly defined psychosis in our sample. SCZ-PGS explained the highest percentage of the variance of psychotic disorders in the liability scale (R^2 = 8.274%; $p < 2x10^{-16}$), followed by BD-PGS (R^2 = 2.414%; $p < 2x10^{-16}$) and MDD-PGS (R^2 = 0.268%; $p = 6.60x10^{-04}$).
We observed an increase in the case-control ratio in progressively higher quartile categories using all PGSs. Compared with individuals in the first quartile, those at the highest quartile had an OR for psychotic disorder risk of 6.037 (95% CI 4.847-7.518) for SCZ-PGS, 2.511 (95% CI 2.048-3.078) for BD-PGS, and 1.302 (95% CI 1.066-1.590) for MDD-PGS.
We assessed PGS contribution to psychotic disorders risk stratified by sex and statistically compared prediction in males and females using a bootstrap resampling approach. All PGS explained significantly more variance in psychotic disorders risk in males than in females (SCZ-PGS: $p < 2.2x10^{-16}$; BD-PGS: $p < 2.2x10^{-16}$; MDD-PGS: $p < 2.2x10^{-16}$)
Our results confirm the shared genetic architecture across psychotic disorders and demonstrate sex-dependent differences in the vulnerability to psychotic disorders. The sex-stratified results are in line with recent findings suggesting that sex differences in the genetic architecture of neuropsychiatric and behavioural traits exist but are small and polygenic (Martin et al. 2021; Blokland et al. 2022). Understanding the genetic basis of sex differences in psychiatric disorders, is crucial for developing sex-stratified diagnostics and therapeutics and for paving the way for precision medicine.

Study of alternative splicing events from RNA-seq in multiplex bipolar families

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Bipolar disorder (BD) is an extremely polygenic disorder. Alternative splicing (AS) events may play a role in BD as result of multiple risk alleles. We used RNA sequencing (RNA-seq) to identify AS

alterations that may pinpoint novel molecular mechanisms contributing to BD's pathophysiology. RNA-seq was performed on total mRNA extracted from lymphoblastoid cell lines of 31 individuals from eight multiplex families (15 BD; 16 unaffected). AS analysis was performed comparing affection status using VAST-TOOLS (VT), rMATS (RM) and MOCCASIN (MCC). Gene lists with significant AS events underwent a gene-based analysis (GBA) in MAGMA using GWAS summary statistics of ten psychiatric disorders. For each gene list an Over Representation Analysis (ORA) was performed with a posterior validation of the significant categories via MAGMA gene-set analysis (GSA). Top AS genes from MCC were selected based on: >30 reads/individual/event, brain expression, P(GBA-BD-GWAS)<0.05. AS events were identified with differential Percentage-Spliced-In (dPSI)>5% and P-value<0.05, with MCC detecting most of AS events (N=2,182). There were no shared AS events amongst the three tools. GSA showed association of MCC AS genes with BD and schizophrenia (SCZ) (P<0.05), that under ORA revealed 26 shared significant categories amongst the two phenotypes, pointing to aggressive behaviours. From MCC top genes, DOCK2 emerged as putative candidate gene for BD, showing a higher skipping event (exon 32 and 33) in BD patients. We observed high variability of AS event detection among computational approaches, emphasizing the importance of employing multiple tools to comprehensively uncover AS events. MCC identifies complex AS events associated with BD and SCZ, whose validated categories from ORA point to aggressive behaviours, a common trait in BD and SCZ. DOCK2, a top AS gene from MCC, maps to a GWAS hit for BD and is expressed exclusively in microglia in brain, mediating inflammation.

Atypical predictive processing in the inferior colliculus of the valproic acid-induced rat model of Autism

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by restricted, repetitive interests, and atypical sensory perception. Atypical sensory processing would compromise environmental experience, hindering the acquisition of progressively more elaborate cognitive abilities. Particularly, when stimuli (e.g. sounds) and contexts (e.g. social) become dynamic and unpredictable. The predictive coding theory frames these symptoms. It postulates that the brain constantly compares prior predictions with upcoming sensory information. In case of mismatch, a prediction error arises, updating the predictions to the new environmental experience, facilitating adaptive behavior. The predictive coding theory of ASD suggests an atypical predictive processing of sensory stimuli, compromising adaptability to non-routine events. To study atypical predictability in ASD, autistic traits were induced to pups born of pregnant rats that received an i.p injection of valproic acid solution (400 mg/kg) at the gestational day 12. Female and male prepubertal (PD30-45) and adult (PD65-120) animals were included, addressing developmental and sex biases. We studied the predictive processing of auditory stimuli at the subcortical level, recording single neurons in the lemniscal and nonlemniscal inferior colliculus. Five auditory conditions were presented: A classical oddball paradigm was used to study mismatch negativity; the brain response to the disruption of the regularity, and control sequences were used to further analyse predictive indexes. Results support the notion of unusual predictive processing in ASD, which could explain limited adaptive behaviour in unexpected situations.

Impact of gonadal and genetic sex on hippocampal function

Pablo Méndez, Rut de la Vega Ruiz, Alicia Hérnández-Vivanco, Alberto Montes-Mellado

Pablo Méndez will describe the impact of biological sex determinants (hormones and chromosomes) in the hippocampus, a brain region essential for memory. He will present data using optogenetic-coupled electrophysiology, fiber photometry and in vivo electrophysiology with silicon probes showing how gonadal and brain synthesized hormones regulate at the synaptic, cellular and network levels the activity of genetically defined population of inhibitory GABAergic neurons. He will talk about how sexual differentiation of the brain determines sex-hormonal regulation of hippocampal function.
He will also present data showing how cell-type specific sex differences in transcriptional profile of hippocampal excitatory and inhibitory neurons could help us to understand sex-biased neurodevelopmental disorders.

Doublesex and mab-3 related transcription factor 2 (Dmrt2) regulates the development of cingulate cortex neurons.

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Sexual differences in behaviour emerge from sex-specific molecular and connectivity brain conformations. The genetic factors involved in the sexual differentiation of the nervous system are poorly understood, but they could become vulnerability or protective factors in the etiology of mental disorders. The family of *doublesex*/*mab-3* related transcription factors (DMRT) are conserved sexual regulators of sexually dimorphic traits across distant species, however the DMRTs' contribution in mammalian brain sexual differentiation has not been approached in depth. In the mammalian nervous system, only the DMA subfamily (*Dmrt3*/*4*/*5*) has been studied. Here, we show an unprecedented role for another member of the family, *Dmrt2*, in proliferation, specification and connectivity of cingulate cortical neurons.
Dmrt2 is broadly expressed in the mouse brain where is influenced by developmental time and sex. In the cingulate cortex (Cg), *Dmrt2* is present in layer VI glutamatergic corticothalamic projecting neurons (CThPN) from early embryo until post-natal animals. By quantifying the distributions of GFP-*in utero* electroporated Cg cells, *Dmrt2* mRNA reduction leads to embryonic progenitors' premature exit from cell cycle and the concomitant reduction of Cg cellular density in layer V and VI. *Dmrt2* downregulation also affects the accurate migration and fasciculation of cingulate CThPN. At late embryos (E18.5), *Dmrt2* silencing causes the downregulation of neurotransmitter transport, neuron projection or synaptic genes (such as *Ptchd1*, *Gabra5*, *Cacna1f* or *Slc6a4*), whereas immune response, cell cycle and cell-cell adhesion related transcripts are increased (*Ccl5*, *Lgals3*, *Rspo1*, *Cdk1*, *Cyclin B1* or *Notch1*). All described phenotypes are more noticeable in males than in females, where *Dmrt2* shows a higher expression level at mid-gestation (E13.5).
Overall, our study suggests a novel role for *Dmrt2* in any mammalian nervous system in the control of cortical developmental processes and circuit maintenance. In human, 9p chromosomal deletion in DMRT1/2/3 is associated with mental disability. The functions described in our study could uncover a possible mechanistic link between the function of a member of the conserved family of DMRTs in sexual susceptibility to mental disorders.

Poster session

A transgenesis system for understanding enhancer function and disease during human cortical development

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The precise spatiotemporal control of gene expression by enhancer elements is crucial during cortical development. In fact, mutations affecting their activity or target gene interactions have been largely associated with neurodevelopmental disorders. Despite the epigenetic identification of more than 50.000 sequences with regulatory potential active in the embryonic human cortex, investigating their functions and the effects of pathogenic variants is hampered by the genetic divergence of humans with classical animal models, technical limitations of enhancer transgenesis assays and ethical concerns for the requirement of large animal numbers. To circumvent this, we have developed a recombination-based toolbox that enables enhancer screening in human iPSC-derived brain organoids. Our approach allows single-copy integration of an enhancer reporter cassette in defined safe harbor genomic loci. We have applied this methodology to study enhancers associated with neuropathogenic variants and related to well-known human neurodevelopmental genes. For one of these genes, the TBR2 transcription factor, we aim to characterize its complete cis-regulatory code to understand how a large genomic inversion spanning *TBR2* regulatory landscape leads to the severe brain malformation reported in patients. Through epigenetic profiling of our brain organoids, we have identified 20 candidate cis-regulatory elements in the *TBR2* locus, which show dynamic chromatin accessibility changes along development. We believe that this methodology will help sharpen the study of enhancer elements and their role during cortical development.

Methylation profile of Williams-Beuren Syndrome in brain and cardiac fetal samples

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Williams-Beuren syndrome (WBS) is a rare genetic disorder caused by the heterozygous deletion of 1.55-1.83Mb at the 7q11.23 region. The deletion of 28-30 genes results in a myriad of phenotypical features with major defects at neurodevelopmental and cardiac levels. Cardiac defects, mainly supravalvular aortic estenosis and pulmonary arterial estenosis, have been attributed mostly to the down regulation of the *ELN* gene, encoding elastine, a component of the elastic fibers. Neurodevelopmental defects such as intellectual disability, autism spectrum disorder and hypersociability are yet to be clearly associated with a particular gene or mechanism. Some studies are pointing to the participation of the *GTF2I* gene family in the cognitive phenotype. The aim of this work is to analyze the epigenetic profile of aortic and neuronal cells during the fetal development of WBS patient samples from biobanks. Many of the genes of the affected region can be linked to epigenetic regulation; for instance, *BAZ1B, BCL7B* and *BUD23* encode proteins related to chromatin remodeling. Other transcriptomic assays in neural *in vitro* models have shown differentially expressed genes from the DNA methyltransferase and histone deacetylase families. Previous

methylation assays on WBS patients have shown specific methylation patterns. However, these studies were limited to blood samples or adult autopsy brain samples. In this study, we focused on the methylation profile of fetal development. We obtained DNA from 3 controls and 1 WBS brain and aorta fetal samples of gestational week 22, and a second WBS aorta sample of 33 gestational weeks (no matched controls available). The bisulfite conversion was performed with the Zymo EZ DNA Methylation Kit and the DNA methylation profiles with the Human Methylation microarray (Infinium Methylation EPICv2 BeadChip, Illumina). Raw Illumina microarray data have been processed with ChAMP R package by following quality control standards. We are currently investigating differentially methylated positions (DMPs) and regions (DMRs) in brain tissue between the WBS and control samples following an epigenome wide approach. Preliminary results show 3293 CpG sites hypomethylated and 6736 CpG sites hypermethylated in the prefrontal cortex of the WBS patient compared to controls (adjusted p-value <0.05). The project was approved by the corresponding Ethical Committees and samples were obtained from the Biobank of the Vall d'Hebron Univesity Hospital (Barcelona). Funding: PID2021-128215OB-I00, MICIU/AEI/10.13039/501100011033/, "FEDER/UE", RYC-2017-21636, RYC2021-033573-I, MICIU/AEI/10.13039/501100011033, PRE2022-102681, PRE2022-103833 and PRE2022-105427.

Can an early-life stress and gonadal hormones impact the reward systems? a study on ABA rats

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Anorexia nervosa (AN) is a psychiatric illness characterised by disturbed eating and selfstarvation.
Its development can be altered by a chronic stressor such as maternal separation (MS) in the postnatal period. Results from a previous study revealed a synergistic mechanism between early MS and a paradigm used to mimic anorectic conditions, the activity-based anorexia (ABA) rat model. In the ABA model, physical activity and restricted food intake provide symptoms like hyperactivity and weight loss. Gonadectomized (GDX) rats of both sexes were exposed to the two paradigms to investigate the role of sex hormones in determining the sexual dimorphic effect of MS, ABA, and the combination of the two stressors. We evaluated anxiety-like behaviors, ABA-related parameters, the reward system, specifically dopamine (DA), serotonin (5-HT), and orexin (ORX) circuits, known to regulate food consumption and locomotor activity.
The results showed that GDX-ABA rats of both sexes displayed hyperactivity and a reduction in anxiety-like behavior compared to controls. Moreover, GDX rats with MSABA revealed sex-dependent alteration of the behavioral phenotype. The immunohistochemical analysis of the circuits linked to the reward system and food consumption seems to show an alteration based on the nuclei considered. In particular, 5-HT and DA system seem to be more affected by the stress event of early MS. On the contrary, the analysis suggests that ORX circuits could be more altered when the stressor is the ABA protocol.
The sexually distinct outcomes studied are probably linked to a different effect mediated by sex steroids on behaviour and reward systems.

Dmrt3 expression in the mouse hindbrain reveals a novel sexually dimorphic nucleus

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The mammalian brain consists of numerous neural circuits interplaying in the modulation of sensory and cognitive functions. Both invertebrates and vertebrates show sexual differences in terms of molecular mechanisms and connectivity; hence, a different response to an identical stimulus may emerge from these brain's sex-specific traits. Nonetheless, the molecular mechanisms underlying these differences and how subtle alterations impinge on behaviour and in the development of mental disorders are barely known. Traditionally, the generation and maintenance of sexual differences in mammals have been exclusively attributed to sex hormones, but, additionally, genetic and epigenetic mechanisms might be acting in a cell-autonomous manner to complement or balance the effect of sex hormones. In this context, the DMRT (Doublesex and Mab-3 Related Transcription Factor) family of transcription factors (TFs) emerged as key, and conserved, regulators of sex differentiation in phylogenetically distant species, guiding the development of sex-specific traits throughout the organism. DMDs/DMRTs in C. elegans confer sex identity to its nervous system through controlling neurogenesis, sex-specific gene expression levels and axonal morphology among others. But a similar role in the mammalian brain remains to be studied. In this work, we describe the comprehensive expression pattern of Dmrt3 (a DMA-DMRT subfamily member) in the female and male mouse brain from early embryonic stages throughout life. We have identified a novel Dmrt3 site of expression in the gigantocellular reticular nucleus (GRN) of the hindbrain. Together with the previously characterized dB4 interneurons, we have identified Dmrt3(+) neurons in the hindbrain to be mostly glycinergic interneurons. The screening of Dmrt3 expression, comparing male and female late embryos, reveals a novel site of sexual dimorphism at the GRN where we observed higher counts of Dmrt3-expressing cells in females than males. The different Dmrt3 counts correlated with higher Dmrt3 mRNA levels in wildtype females. To inquire about the origin of this novel dimorphism, we measured Dmrt3 mRNA in the Four Core Genotype (FCG) model, where sex chromosomes and sex hormones are decoupled. As expected, the XX group showed higher levels of Dmrt3 mRNA in both wildtype and FCG backgrounds. However, in the FCG background, no clear effects of the gonadal or genetic components were observed in the other three conditions (XXSry, XY- and XY-Sry), in which Dmrt3 mRNA levels were lower. Besides Dmrt3's role in cortical neurogenesis and cortical patterning, Dmrt3 is involved in the specification of dI6 interneurons of the spinal cord. Hence, we propose that Dmrt3 could be involved in the specification of hindbrain neuronal populations and in the sex-specific numbers in cooperation with sexual factors. To test this hypothesis, we will use a nestinCre;Dmrt3Flox conditional mouse model to transcriptionally profile GRN neurons in the presence and absence of Dmrt3 in relation to sex. Our research sheds light onto a new anatomical point of sexual dimorphism in the mouse hindbrain and the potential implication of a conserved Dmrt TF in the establishment of sexual differences.

Analysis of sexual dimorphism in the expression of Pou3f2 in the developmental brain

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Investigating the molecular mechanisms that govern brain development across different sexes is crucial for understanding the origins of neurodevelopmental disorders. The transcription factor Pou3f2, which plays a pivotal role in neural development, has been associated with various mental health conditions, including schizophrenia and autism spectrum disorders. By employing sophisticated molecular

techniques, we have examined the expression dynamics of Pou3f2 throughout brain development, illuminating potential sex-specific regulatory mechanisms. Our research offers significant insights into the molecular bases of sex differences in brain function and establishes a foundation for further studies into the etiology of neurodevelopmental disorders.

The Madrid Manic Group (MadManic): a novel Spanish cohort for multidisciplinary studies in translational psychiatry.

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The Madrid Manic Group (MadManic) is a novel cohort from Spain for the study of psychiatric disorders. The recruitment was initiated in 2022. This cohort includes patients with bipolar disorder (BD), suicide attempts (SUI), schizophrenia (SCZ), and control subjects. The aims of this established study group are: i) Advance our knowledge in the genetics implicated in psychotic disorders and suicide; ii) Develop pioneering and multidisciplinary methods for the identification of psychiatric patients at-high-risk for suicide; iii) Identify genetic markers and transcriptomic signatures for treatment response of mood stabilizers in BD.
Psychiatric patients are clinically assessed at following hospitals and clinical units in the metropolitan area of Madrid: Hospital Fundación Jiménez Díaz and the related clinical units of Pontones and Quintana, Hospital General de Villalba, Hospital Rey Juan Carlos, and Hospital Infanta Elena de Valdemoro. Controls are recruited at occupational medicine services of the Spanish National Research Council (CSIC). Individuals provide a blood sample for extractions of DNA, RNA, and plasma in a centralized biobank at Molecular Biology Centre Severo Ochoa (CBMSO). The biospecimens from the MadManic cohort are currently used for the following genomic arrays: i) Axiom Spanish Array (~758,000 genotyped SNPs/individual); ii) blood-derived mRNAs and lncRNAs sequencing via NovaSeq X plus (100 million reads/individual; 15Gb/sample); iii) Infinium MethylationEPIC v2.0 BeadChip (935,000 CpG sites/individual).
The phenotyping is based on a clinical electronic platform and digital phenotyping:

1) Clinical data and scales recorded electronically and centralised in the *MeMind* platform (>2,000 items/patient), which include sociodemographic records, sleep patterns, comorbidities, drug dependence, hospitalizations, psychosis, suicide behaviours, pharmacological treatments (regime, side effects, and drug response), the ALDA scale to quantify improvements during long-term treatments with lithium, functional impairment scales (WHODAS 2.0, GAF, and CGI), chronotype (uMCTQ), childhood trauma (CTQ), laterality (Edinburg), personality scales (BFI-2 and Millon), and creativity (Dollinger). The suicide patients are enrolled in the SMARTcrisis 3.0 clinical trial, assessing suicide-related traits.
2) Ecological momentary assessments are carried out via smartphone applications: i) *MeMind* (www.memind.net), that monitor suicide risk and assess suicidality, quality of sleep, negative feelings, etc; ii) Evidence-based Behaviour (eB2) (https://eb2.tech), which is based on a passive interaction with patients collecting continuously data via native sensors registering smartphone usage patterns (time of use of the device, phone call duration), mobility (location, distance travelled, and speed), physical activity, sleep quality, and prosody (elements of speech such as intonation, tone, stress, and rhythm). Controls provide demographic information and several behavioural scales (CTQ, uMCTQ, Edinburgh, BFI-2, Dollinger, suicide behaviours).
The MadManic has generated a novel international resource for the study of psychiatric disorders, which includes a recent recruitment (2022-24) of 1,700 subjects (450 BD patients, 350 SUI, and 900 controls) with both biospecimens and clinical records. The biobank includes >5,000 biospecimens (DNAs, RNAs, and plasma). Schizophrenia patients are currently under recruitment. The MadManic cohort sums also a previous DNA collection (1996-2010) of patients with several psychiatric diagnoses (~1,000). The genomic and transcriptomic arrays are being generated for the following genomic arrays: i) Genome-wide SNP data for 5,900 individuals: 528 BD, 819 SUI, 200 SCZ, and 4,350 controls (MadManic+BNADN); ii) FastQ data from RNA sequencing of 700 subjects (BD and controls); iii) Methylome data for 200 individuals (100 BD and 100 controls).
We present a novel study group in psychiatry that will deliver novel strategies for medical interventions in primary unsolved clinical problems by combining genome-wide data, computational pipelines, digital phenotyping and machine learning approaches. The MadManic will make a significant contribution to large international efforts in psychiatric genetics, pharmacogenomics studies, and clinical presentations of BD. We are implementing novel strategies for the identification of psychiatric patients at high-risk for suicide for individual clinical interventions.

RNF10 **role in synaptic plasticity and Alzheimer Disease**

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RNF10 is a protein with multiple cellular functions. It has been described as a E3-Ubiquitin ligase, with a key quality-control role during the initiation of translation in the early regulatory ribosome ubiquitylation (RQC). In addition, RNF10 plays in neurons a role as a synaptonuclear messenger, involved in the NMDA receptors (NMDAR) signaling cascade. RNF10 binds to the C-terminal region of the GluN2A subunit of the NMDAR and itstranslocation from the synapse to the nucleus translates synaptic NMDAR activation into gene expression changes. RNF10 is crucial in the physiology of the neurons, being involved in LTP-dependent modulation of dendritic spine morphology and in shaping of the dendrite's architecture in hippocampal neurons. Preliminary data show a potential involvement of RNF10 in Alzheimer's Disease (AD) pathogenesis. Amyloid beta oligomers, that are responsible for AD synaptic failure, promote RNF10 trafficking from the synapse to the nucleus, suggesting that RNF10 can be a mediator of Amyloid beta synaptotoxicity. To investigate the downstream effects of Amyloid beta-

triggered RNF10 synapse-to-nucleus communication, we investigated RNF10 function as transcription factor and as regulator of ubiquitination in early translation. To this, we performed RNA-seq analysis using RNF10-silenced hippocampal neurons exposed to either Amyloid beta oligomers or to a synaptic stimulation protocol. Using the same experimental paradigm, we performed SUnSET experiments to evaluate the effect of RNF10 in early translation and specific assays to detect the levels of ubiquitination. The results provided a global picture of RNF10 function in physiological synaptic plasticity phenomena and in AD pathology.

Functional characterization of enhancers controlling the expression of human neurodevelopmental genes in organoid models

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Brain cortex development hinges upon the activity of genes exhibiting dynamic and tissue/cell-specific expression patterns. Typically, their transcription is governed by combinations of cis-regulatory elements (CREs) such as enhancers, repressors, and silencers. Mutations in CREs are associated with congenital brain defects and human brain evolution.

Although the availability of chromatin epigenetic profiles has facilitated the identification of potential CREs on a genomic scale, their functional characterization requires intricate functional genomic assays, often challenging to execute in animal models. Moreover, the evolutionary disparities in the human non-coding genome and cortex development hamper the study of human neurodevelopmental gene regulation and of brain-pathological regulatory mutations.

We address this challenge by using human brain organoids as model system and developing novel recombination- mediated enhancer transgenesis approaches exploiting the properties of the PhiC31 or Bxb1 integrases. These methods yield a single-copy integration of a reporter construct designed to minimize genome position effects, and eliminate the insert copy number variations of classic transgenesis. Additionally, they are largely independent of the size of the tested enhancer. In contrast, PhiC31 and Bxb1 transgenesis differ in efficiency and specificity. PhiC31 recombination emerges as the most efficient method, enabling the rapid generation and analysis of enhancer reporter iPSC lines. Conversely, Bxb1 integrates the transgenic construct at a targeted position, offering the most reliable mean to compare the activity of different enhancer alleles, thereby facilitating the study of pathological enhancer polymorphisms.

Combining epigenetic, chromatin 3D architecture and GWAS databases we identified candidate regulatory sequences that: (1) exhibit active enhancer epigenetic marks in the human foetal cortex, (2) are located in the regulatory domain of known neurodevelopmental genes, and (3) are associated with polymorphisms described in patients with brain disorders/malformations. Our findings suggest that combining our recombination transgenesis approaches with the use of region-specific brain organoids may offer a potent approach to characterize the activity of these candidate enhancers and the effect of their associated pathological mutations.

Deciphering the role of the chromatin remodeler *CHD2* in human cortex development through brain organoid analysis

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The diversity of neurons composing the human cerebral cortex arise during embryonic development through a process called neurogenesis. Epigenetic modifications play an essential role in its regulation by controlling the activity of neurodevelopmental genes through different and interrelated mechanisms.
CHD2 is a chromatin remodeler whose mutations in humans have been associated with different brain disorders/malformations, including epileptic encephalopathies, autism, cognitive impairment, microcephaly etc. However, the manifestation and severity of these disorders vary widely among patients, possibly influenced by different types of inactivating mutations affecting *CHD2*. While *Chd2* inactivation in mice alters cortical neurogenesis, it fails to fully replicate the spectrum of clinical phenotypes observed in patients. Moreover, the precise genetic and epigenetic mechanisms through which CHD2 regulates cortical development remain largely unexplored, as does the underlying cause of the phenotypic diversity observed in patients.
The analysis of clinical data suggests that heterozygous *CHD2* mutations disrupting its chromatin remodeling activity but sparing the chromatin binding domains correlate with increased severity of pathological phenotypes compared to mutations affecting all its functional domains. One plausible explanation is that these truncating mutations produce a shortened CHD2 protein that interferes with the function of the wild-type allele.
Besides, the human *CHD2* locus is predicted to encode transcriptional isoforms not described in mice. This may account, at least in part, for the partial recapitulation of *CHD2* pathological phenotypes in mouse models. We observed that these human-specific isoforms encode a truncated CHD2 variant, retaining the chromatin-binding domain but lacking chromatin remodeling activity. Consequently, an imbalance in isoform activity due to mutations specifically disrupting the full-length CHD2 variation may contribute to the phenotypic variability observed in patients.
To delve deeper into these mechanisms, we characterized the human *CHD2* isoform expression in iPSC-derived cortical organoids, which faithfully recapitulate the cortical neurogenic process. Furthermore, utilizing CRISPR/Cas9 genome editing, we generated iPSC lines harboring several *CHD2* mutations, mimicking those found in patients. Subsequent analysis of cortical organoids derived from these mutant iPSC lines unveiled their impact on organoid morphology and neural precursor populations, shedding light on the intricate role of *CHD2* in cortical development and disease pathology.

Late developmental depletion of Cux1 in cortical neurons alters nuclear and dendritic architecture

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Cux1 is a transcription factor (TF) associated with various neurodevelopmental and mental diseases, such as epilepsy and autism spectrum disorders. Within the cerebral cortex, Cux1 is predominantly expressed in the upper layers. In these neurons, Cux1, together with Cux2, governs several aspects of neuronal identity and connectivity. However, the functions of these TFs in mature neurons remain barely explored. To study the role of Cux1 in the homeostatic maintenance of cortical neurons, we generated a floxed Cux1 allele to conditionally knockout with CRE (Cux1 cKO). Using in utero electroporation, we introduced tamoxifen-dependent CRE plasmids in L2/3 neurons and induced the deletion of Cux1 at postnatal day (P) 21, when neuronal migration and the acquisition of neuronal identity is completed. The effect of Cux1 deletion was evaluated at different sequential stages: P31, P37, P60, and P120.

The analysis of the electroporated neurons at P31 shows that Cux1 depletion alters chromatin condensation and nuclear architecture. At P37, these changes in the nucleus are reversed in many neurons, but others are lost. There is a decrease in electroporated cell density between P31 and P37. We also observe hyperactivation of microglia at P31, also indicating that a subset of Cux1-dependent neurons are cleared by microglial due to Cux1 deficiency. Neuronal numbers remain stable thereafter, as shown by the analysis of P60 and P120 animals, albeit a population of neurons still displays an aberrantly enlarged nucleus with chromatin in the periphery. Moreover, at P37, Cux1-deficient neurons showed a reduction in the density and complexity of dendritic spines compared to wild-type animals, but at P60, they show double the number of dendritic spines than WTs. This increase in dendritic spines has been observed in neurodegeneration and appears as an attempt to compensate for the neuronal loss.
Thus, neuronal viability, nuclear morphology, and dendritic spines depend upon Cux1 expression. We theorize that the observed phenotypes relate to the roles of CUX1 protein as nuclear matrix attachment proteins (MARS) and chromatin structure. Taken together, our findings could help to shed light on the role of Cux1 in mature neurons and highlight the importance of investigating the function of TFs in late development.

Androgens support hippocampal-dependent memory and regulate dorsal ca1 hippocampal oscillations and excitatory-inhibitory circuits.

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The influence of male gonadal sex hormones on cognition has been extensively documented during the past decades. The actions of sex hormones in male cognition have been partially attributed to the regulation of gene expression, synaptic transmission and neuronal physiology in the hippocampus, a brain region involved in learning, memory and spatial navigation. Hippocampal function relies in the structured connectivity between diverse groups of excitatory and inhibitory neurons. The coordinated activity of excitatory/inhibitory microcircuits gives rise to different forms of network oscillations (theta, gamma oscillations and sharp wave ripples), which underlie different aspects of information acquisition, consolidation and recall.
Experimentally, the effects of male sex hormones are usually tested through orchidectomy and exogenous androgen administration in rodents. These procedures have been proven to have an effect on spatial learning and reference memory tasks, although the cellular and network mechanisms mediating these effects remain still largely unknown. Here, we aim to study the circuit and network mechanisms in the hippocampus that account for cognitive alterations as a consequence of castration in male mice and the potential recovery of these functions through exogenous replacement of androgens.
First, we conducted experiments to asses hippocampal-dependent memory in three groups of male mice: sham gonadectomy and empty silastic tube (Sham); orchidectomy and empty silastic tube (ORX); gonadectomy and DHT filled silastic tube (ORX+DHT). We tested spatial learning and memory in a four-arm circular shaped maze (P-Maze) and emotion-guided spatial learning in a four-day contextual fear conditioning (CFC) paradigm. Secondly, we performed electrophysiological recordings of local field potentials (LFP) in the same groups of awake behaving mice to determine the effects of ORX and DHT on dorsal CA1 hippocampal network activity. To evaluate the effects of castration and DHT replacement on hippocampal oscillations, LFP from different CA1 layers was processed and theta and gamma oscillations were extracted and analyzed by using custom-made MatLab code. The detection of sharp-wave ripples (SWR) was carried out by computational means in order to avoid biased detection of these events. Further analyses of SWR will be made through custom-made MatLab code. To further study the cellular mechanisms responsible for the observed effects on behavior, we characterize electrophysiological features of CA1 dorsal hippocampus excitatory/inhibitory microcircuits by performing patch clamp recordings in acutely prepared hippocampal slices from Sham and ORX male

mice. Finally, transcriptomics analyses were performed to determine androgen receptor expression in different neuronal types in the hippocampus.
Ongoing analyses of mice behavior in the P-Maze showed that all groups similarly improved their performance, indicating similar learning within same-day sessions. However, differences in performance were evident when comparing learning across days. ORX mice showed no change in learning increase across days, while both Sham and ORX+DHT groups displayed increased learning across days. This suggests that while spatial coding during the same day's task might be conserved, retention or long term recovery of spatial information is altered as a consequence of castration and is recovered by the exogenous administration of DHT. Results of contextual fear conditioning paradigm revealed that ORX and DHT did not impact the expression of the conditioned response (freezing). However, ORX mice showed a tendency towards poorer discrimination between safe and threating contexts, while Sham and ORX+DHT mice efficiently discriminate as revealed by the ratio of freezing behavior expression in both contexts.
Hippocampal LFP recordings in awake behaving mice unveiled differences in network activity between Sham and ORX male mice. Preliminary analysis showed differences in mean power in theta and gamma band frequencies in ORX mice compared with intact male mice, suggesting ORX effects on hippocampal oscillations. We are currently analyzing fast frequency oscillations, i.e. sharp-wave ripples (SWRs), due to their role in memory consolidation. Taking into account the memory retention impairments observed in ORX mice in the P-Maze, SWRs are good candidates to mediate ORX effects on hippocampal-dependent behavior. Additional hippocampal LFP recordings are being conducted and analyzed in the replacement condition (ORX+DHT) to evaluate if DHT could restore these alterations.
Ex vivo patch clamp electrophysiological recordings in acutely prepared hippocampal slices showed that ORX and pharmacological blockade of DHT synthesis with systemic treatment with the 5α-reductase inhibitor finasteride cause similar alterations in CA1 synaptic inhibition onto CA1 pyramidal neurons (sIPSCs). The effect of ORX in CA1 Excitatory/Inhibitory microcircuits is in line with our analysis of transcriptomic data that suggest that the mRNA of androgen receptor (AR), the cellular receptor for DHT and T, is expressed in different proportions of both excitatory (CA1, CA3, GC) and inhibitory neurons (Sst, Pvalb, Lamp5, Scng, Vip).
Altogether, these results show that peripheral androgens support hippocampal-dependent long-term spatial memory retention in male mice. ORX and suppression of DHT synthesis affect the physiology of hippocampal microcircuits formed by excitatory and inhibitory neurons, which express the cellular receptor for androgens. The alteration of excitatory and inhibitory activity in hippocampal may in turn impact the functionality of hippocampal oscillations and provide a potential mechanistic link to spatial memory retention deficits.

Role of *DMRTA1 and DMRTA2* genes in the genetic susceptibility to ADHD

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Recent studies have shed light on the fundamental roles of *DMRT* (*doublesex* and *mab-3* related transcription factors) gene family in sexual differentiation and brain development. These genes encode transcription factors (TFs) that control sexual differentiation by defining neuron numbers, cellular identity, and connectivity. Among them, *DMRTA1* (*Dmrt4* in mouse) and *DMRTA2* (*Dmrt5* in mouse*)* are notable for their role in cortical development. In humans, homozygous mutations in *DMRTA2* cause a rare and severe condition characterized by microcephaly and lissencephaly, resembling phenotypes associated

with mutations in *RELN, LIS1*, and *TUBA1A* genes. In addition to cortical expression, preliminary data from our laboratory shows that *Dmrta2* is expressed in the ventral midbrain, where it suppresses transcriptional differences between male and female embryos. Moreover, both, in cortical and in ventral midbrain progenitors *Dmrta2* represses the expression of *Dmrta1,* and in *Dmrta2* mutants, *Dmrta1* is ectopically expressed. We used GWAS summary statistics from the Psychiatric Genomics Consortium (PGC) to assess the impact of single nucleotide polymorphisms (SNPs) in seven *DMRT* genes across ten psychiatric disorders. We found significant associations with Attention-Deficit/Hyperactivity Disorder (ADHD) driven by SNPs located on *DMRTA1* and *DMRTA2* regulatory regions. The significant *DMRTA1* SNPs at promoter constitute a block of linkage disequilibrium, in which we identified a risk haplotype (GGCG) for ADHD, being the rarer haplotype (4%) in non-Finnish European populations. To investigate the functional implications of these genetic associations, we performed luciferase assays in neuroblastoma cells of 4 haplotype blocks, observing higher expression levels in the ADHD-associated H4 haplotype compared to the common haplotype H1 (GGCA). We also analysed imaging genomics data from the ENIGMA consortium, which study found that genetic variants in *DMRTA2* affect numerous brain structures, whereas *DMRTA1* variants are specifically linked to the cortical regions of precuneus and lingual surface areas. Both regions are involved in the perception and processing of stimuli which are crucial for cognitive functions disrupted in ADHD. Our hypothesis suggests that variants in *DMRTA1* and *DMRTA2* regions contribute to ADHD by altering their expression with potential sex-specific effects, while also playing conserved roles in midbrain dopaminergic and cortical neuron development and specification. We plan to investigate their contributions using human midbrain (hMO) and cortical (hCO) organoids. Together with CRISPR/Cas9 genome editing in human induced Pluripotent Stem Cells (iPSCs) we will study *DMRTA1* and *DMRTA2* null strains using human brain organoids. In summary, our study will elucidate the genetic underpinnings of *DMRTA1* and *DMRTA2* in brain development and their potential contribution to ADHD. We hypothesize that altered expression of *DMRTA1* may contribute also to the sex bias observed in ADHD through sex-dependent expression changes, with significant effects on neuronal differentiation and connectivity in the midbrain and cerebral cortex.

Probing and analyzing the activity of brain organoids with advanced high-density microchip technology

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Brain organoids hold immense promise for studying complex neurological disorders, but dissecting their functional phenotypes remains a hurdle. Here, we present a powerful approach using high-density microelectrode arrays (HD-MEAs) to record and analyze electrical activity in brain organoids. This method enables us to probe the functional organization of these 3D structures at high resolution. We demonstrate the utility of HD-MEA by characterizing 5-month-old brain organoids derived from a healthy cell line. The dense electrode array allows for simultaneous recordings from thousands of sites with excellent signal-to-noise, revealing the firing patterns of individual neurons within the network. To showcase its potential for drug discovery, we monitored organoid activity before and after treatment with 4-aminopyridine and cyclothiazide. Our data show dramatic changes in key functional metrics like firing rate and network connectivity upon drug exposure. In conclusion, this study validates HD-MEA technology as a powerful tool to unlock the full potential of brain organoids for unraveling the complexities of neurological disorders and drug development