Hypothalamic cellular changes in neuroendocrine disorders

Human postmortem studies

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English summary

The main goal of this thesis was to investigate neuronal and glial alterations in the hypothalamus of individuals with neuroendocrine disorders, focused on type 2 diabetes (T2DM) and Prader-Willi Syndrome (PWS). In **Chapter 1**, we provided an overview of the glial alterations in two metabolic disorders – obesity and anorexia nervosa. Both overnutrition and undernutrition are associated with notable changes of glial cells, particularly microglia and astrocytes. These alterations culminate in a drastic remodeling of the neurocircuitry that integrates the homeostatic drive of food seeking, learned behavior and hedonic components of feeding. In **Chapter 2**, we deepened into specific hypothalamic alterations associated with PWS, a genetic neurodevelopmental disorder associated with uncontrollable hyperphagia and extreme obesity. We provided an overview of the genetic components of this disorder, abnormalities of endocrine factors, neuropeptides and neurocircuitry altered in this pathology, and the potential contribution of glial cells for the clinical outcomes of the pathology.

Following the introductory chapters, the thesis delves into the investigation of hypothalamic neuronal and glial cells in the context of PWS and T2DM pathophysiology. Special attention is given to brain regions governing circadian rhythm (**Chapter 3**) and energy homeostasis (**Chapters 4**, **5**, **6** and ***7***).

Chapter 3 seeks to elucidate the neuroanatomical basis for circadian misalignments in the context of PWS. First, we investigated whether PWS causative genes are expressed in the murine suprachiasmatic nucleus (SCN), the central master clock, at single cell resolution. We found robust levels of expression of some PWS related genes in the SCN, indicating a role for those genes in SCN timekeeping. Next, we profiled the SCN cellular composition of PWS patients compared to controls. We observed a selective increase in numbers of arginine-vasopressin-immunoreactive (AVP-ir) neurons in PWS subjects compared to controls, associated with reduced astrocytic presence. These data indicate a major neuropeptidergic and numeric imbalance of two of the major pacesetter cellular types of the SCN in PWS. We also found increased glymphatic system activity in the SCN of PWS subjects, potentially emerging as an adaptive response to metabolic waste accumulation. These results provide the first neuroanatomical basis for circadian disturbances in PWS, and suggest that circadian-based therapies (*i.e.* melatonin therapy; timed light exposition) might ameliorate some of PWS-related symptomatology.

Insatiable hunger and morbid obesity are among the most outstanding clinical manifestations of PWS. Numerous studies in animal models and humans demonstrated that appetite-controlling hypothalamic neurons are dysfunctional in PWS, culminating in a metabolically disrupted phenotype. Disruption of these neurons includes abnormal synaptic function and connectivity. To understand the etiopathological basis of this phenomenon, we investigated whether PWS is associated with alterations of abundance of perineuronal nets (PNNs) in **Chapter 4**. Our analysis showed that PNNs are drastically reduced in the hypothalamus of PWS subjects, indicating that neurons in control of metabolism are structurally immature in this disorder. Ultimately, these results provide potential hints of the cellular basis for abnormal connectivity of neurons relevant for feeding behavior and energy expenditure in PWS.

Hypothalamic dysfunction is deemed to be, at least partially, the basis of the neuroendocrine, metabolic and behavioral deviances in PWS. Of genetic etiology, loss of expression of paternally-inherited genes on chromosome 15q11-q13 culminate on the PWS phenotype. Importantly, PWS subgenotypes are recognized and categorized by the extension of the chromosomal deletion; and Type I deletions affect a larger genomic region than type II deletions. Patients with a Type I deletion present enhanced aggressiveness and compulsive-obsessive behaviors in comparison to Type II deletion patients. Our analysis in **Chapter 5** revealed a distinct transcriptional hypothalamic signature between the two subgenotypes, indicating that exacerbation of hypothalamic dysfunction in PWS Type I might underlie the more severe symptomology observed in those individuals. At the cellular level, our analysis revealed worsened white matter microstructure, neuron-neuron synaptic communication and microglial immunosurvilleance in hypothalami of PWS Type I patients. Our evidence also suggests that these neural and glial alterations are, at least partially, due to loss of the gene *Cyfip1*. Taken together, we provided the first line of evidence that comprehensively links hypothalamic malfunctioning to genotypic traits in deletion subtypes, advocating for personalized therapeutic strategies for each subgenotypes.

A growing amount of evidence derived from experiments on rodents has demonstrated the role of hypothalamic dysfunction in the development of T2DM. However, the clinical significance of these findings is yet to be determined. Hypothalamic lipid levels have been implicated in the establishment and progression of metabolic diseases. To understand the relevance of lipid biology during different metabolic perturbations, we investigated

hypothalamic lipid droplets (LDs). LD is the universal organelle for lipid storage and a hydrophobic reactions platform. In **Chapter 6**, our analysis of LD distribution in the mediobasal hypothalamus revealed that the ependymal cells in the third ventricle contain the majority of hypothalamic LD, in both mice and humans. High fat diet feeding and insulin resistance increases LD content in mice, whereas T2DM pathophysiology leads to a decrease in LD in the human hypothalamus. These alterations might impact hypothalamic lipid sensing and metabolism, in both neurons and glia cells. Our data point to a distinct modulation of LD content in both species during metabolic disruption.

In **Chapter 7**, we explored the impact of T2DM pathophysiology on neural and glial cells in the hypothalamic paraventricular nucleus (PVN), one of the most important glucoregulatory centers in the brain. We reported a selective decrease in numbers of oxytocin-containing neurons in the PVN of T2DM subjects, similar to what is observed in obese and PWS patients. Oxytocinergic circuitry has been largely implicated in the feeding behavior and glucose homeostasis in murine models of obesity, however, it remained unexplored in the human T2DM condition. AVP- and corticotrophin releasing hormone-containing neurons numbers were unaffected in T2DM condition. Parallel to these neural changes, we observed astrocytic dysfunction and recruitment of glymphatic system activity in T2DM, but similar microglial immunity profiles among both groups. These results indicate a lack of trophic support and unhealthy microenvironment in the hypothalamus of T2DM subjects. However, the cellular basis for such dysfunction is distinct to other metabolic disorders, to which microglial activation is a fundamental contributor to hypothalamic disruption.

In summary, this thesis provides valuable insights into the complex interplay between neuronal and glial alterations in the context of neuroendocrine disorders, shedding light on potential therapeutic avenues to be adopted in each context.

Nederlandse samenvatting

Het belangrijkste doel van dit proefschrift was het onderzoeken van neuronale en glia veranderingen in de hypothalamus van individuen met neuro-endocriene aandoeningen, gericht op type 2 diabetes (T2DM) en Prader-Willi Syndroom (PWS). In **Hoofdstuk 1** gaven wij een overzicht van de glia veranderingen in twee stofwisselingsstoornissen: obesitas en anorexia nervosa. Zowel overvoeding als ondervoeding worden in verband gebracht met opmerkelijke veranderingen in gliacellen, met name microglia en astrocyten. Deze veranderingen culmineren in een drastische hermodellering van het neurocircuit dat de homeostatische drang van het zoeken naar voedsel, aangeleerd gedrag en hedonistische componenten van voeding integreert. In **Hoofdstuk 2** hebben wij ons verdiept in specifieke hypothalame veranderingen geassocieerd met PWS, een genetische neuropsychiatrische ontwikkelingsstoornis geassocieerd met oncontroleerbare hyperfagie en extreme obesitas. We gaven een overzicht van de genetische componenten van deze aandoening, afwijkingen in endocriene systemen, neuropeptiden en neurocircuits bij deze pathologie, en de potentiële bijdrage van gliacellen aan de klinische uitkomsten van de pathologie.

Na de inleidende hoofdstukken gaat het proefschrift dieper in op het onderzoek van neuronen en gliacellen in de hypothalamus in de context van de pathofysiologie van PWS en T2DM. Speciale aandacht wordt besteed aan hersengebieden die het circadiane ritme (**Hoofdstuk 3**) en de energiehomeostase (**Hoofdstukken 4**, **5**, **6** en **7**) bepalen.

Hoofdstuk 3 probeert de neuroanatomische basis voor circadiane afwijkingen in de context van PWS op te helderen. Eerst hebben wij onderzocht of de PWS-veroorzakende genen tot expressie worden gebracht in de suprachiasmatische kern (SCN) van muizen, de centrale hoofdklok, op basis van een techniek met een resolutie van één enkele cel. We vonden robuuste expressieniveaus van sommige PWS-gerelateerde genen in de SCN, wat wijst op een rol voor die genen in de SCN-klok functie . Vervolgens hebben wij de SCN-cellulaire samenstelling van PWS-patiënten vergeleken met controles. We hebben een selectieve toename waargenomen in het aantal arginine-vasopressine-immunoreactieve (AVP-ir) neuronen bij PWS-proefpersonen vergeleken met controles, geassocieerd met verminderde astrocytaire aanwezigheid. Deze gegevens duiden op een sterke verstoring van de neuropeptiderge en numerieke balans van twee van de belangrijkste gangmakerceltypen van de SCN bij PWS. We vonden ook een

verhoogde activiteit van het glymfatische systeem in de SCN van PWS-patiënten, wat geïnterpreteerd kan worden als een adaptieve reactie op de accumulatie van metabole afval. Deze resultaten vormen de eerste neuroanatomische basis voor circadiane stoornissen bij PWS, en suggereren dat op circadiane gebaseerde therapieën (dat wil zeggen melatoninetherapie; getimede blootstelling aan licht) een deel van de PWS-gerelateerde symptomatologie zouden kunnen verbeteren.

Onverzadigbare honger en morbide obesitas behoren tot de meest opvallende klinische manifestaties van PWS. Talrijke onderzoeken bij diermodellen en bij mensen hebben aangetoond dat neuronen die de eetlust controleren disfunctioneel zijn bij PWS, wat culmineert in het metabool verstoorde fenotype. Verstoring van deze neuronen omvat abnormale synaptische functie en connectiviteit. Om de etiopathologische basis van dit fenomeen te begrijpen, hebben we in **Hoofdstuk 4** onderzocht of PWS geassocieerd is met veranderingen in de perineuronale netten (PNNs). Onze analyse toonde aan dat PNNs drastisch verminderd zijn in de hypothalamus van PWS-patiënten, wat aangeeft dat neuronen die de controle hebben over het metabolisme zijn bij deze aandoening. Uiteindelijk bieden deze resultaten aanwijzingen voor de cellulaire basis voor abnormale connectiviteit van neuronen die relevant zijn voor het voedingsgedrag en het energieverbruik bij PWS.

Er wordt aangenomen dat hypothalame disfunctie, althans gedeeltelijk, de basis is van de neuro-endocriene, metabole en gedrags afwijkingen bij PWS. De genetische etiologie culmineert in het verlies van expressie van vaderlijk overgeërfde genen op chromosoom 15q11-q13 in het PWS-fenotype. Belangrijk is dat PWS-sub-genotypes worden herkend en gecategoriseerd aan de hand van de uitbreiding van de chromosomale deletie. Type I-deleties beïnvloeden een groter genomisch gebied dan type II-deleties. Patiënten met een Type I-deletie vertonen een verhoogde agressiviteit en dwangmatig-obsessief gedrag in vergelijking met Type II-deletiepatiënten. Onze analyse in **Hoofdstuk 5** onthulde een duidelijke transcriptionele hypothalame signatuur tussen de twee subgenotypes, wat aangeeft dat verergering van de hypothalamusdisfunctie bij PWS Type I ten grondslag zou kunnen liggen aan de ernstigere symptomologie die bij deze individuen werd waargenomen. Op cellulair niveau onthulde onze analyse een verslechterde microstructuur van de witte stof, neuron-neuron synaptische communicatie en microgliale immunosurveillance in de hypothalami van PWS Type I-patiënten. Ons onderzoek suggereert ook dat deze neurale en glia veranderingen,

althans gedeeltelijk, te wijten zijn aan het verlies van het gen *Cyfip1*. Alles bij elkaar hebben we het eerste bewijs geleverd die het slecht functioneren van de hypothalamus volledig gekoppeld aan genotypische kenmerken in deletiesubtypen, waarom wij pleiten voor gepersonaliseerde therapeutische strategieën voor elk subgenotype.

Een groeiende hoeveelheid bewijsmateriaal afkomstig uit experimenten met knaagdieren heeft de rol van hypothalame disfunctie bij de ontwikkeling van T2DM aangetoond. De klinische betekenis van deze bevindingen moet echter nog worden vastgesteld. Hypothalame lipidenniveaus zijn betrokken bij het ontstaan en de progressie van stofwisselingsziekten. Om de relevantie van de lipidenbiologie tijdens verschillende metabole verstoringen te begrijpen, hebben wij hypothalame lipidendruppeltjes (LDs) onderzocht. LD is het universele organel voor de opslag van lipiden en een platform voor hydrofobe reacties. In **Hoofdstuk 6** onthulde onze analyse van de LD-distributie in de mediobasale hypothalamus dat de ependymcellen in het derde ventrikel het merendeel van de hypothalame LD bevatten, zowel bij muizen als bij mensen. Voeding met een hoog vetgehalte en insulineresistentie verhogen het LD-gehalte bij muizen, terwijl T2DM-pathofysiologie leidt tot een afname van LD in de menselijke hypothalamus. Deze veranderingen kunnen van invloed zijn op de detectie en het metabolisme van lipiden in de hypothalamus, zowel in neuronen als in gliacellen. Onze gegevens wijzen op een duidelijke modulatie van het LD-gehalte bij beide soorten tijdens metabole verstoring.

In **Hoofdstuk 7** onderzochten we de impact van T2DM-pathofysiologie op neuronen en glia cellen in de hypothalame paraventriculaire kern (PVN), een van de belangrijkste glucoseregulerende centra in de hersenen. We rapporteerden een selectieve afname van het aantal oxytocine-bevattende neuronen in de PVN van T2DM-patiënten, vergelijkbaar met wat wordt waargenomen bij zwaarlijvige en PWS-patiënten. Oxytocinerge circuits zijn betrokken bij het voedingsgedrag en de glucosehomeostase in muizenmodellen van obesitas, maar bleven onontgonnen in de menselijke T2DM-aandoening. Het aantal AVP- en corticotropine releasing hormoon bevattende neuronen werd niet beïnvloed doore de T2DM-toestand. Parallel aan deze neurale veranderingen observeerden we astrocytische disfunctie en rekrutering van glymfatische systeemactiviteit in T2DM, maar vergelijkbare microgliale immuniteitsprofielen tussen beide groepen. Deze resultaten wijzen op een gebrek aan trofische ondersteuning en een ongezonde micro-omgeving in de hypothalamus van T2DM-patiënten. De cellulaire basis voor een dergelijke disfunctie

verschilt echter van die van andere stofwisselingsstoornissen, waarbij microgliale activering een fundamentele bijdrage levert aan de verstoring van de hypothalamus.

Samenvattend biedt dit proefschrift waardevolle inzichten in de complexe wisselwerking tussen neuronale en glia veranderingen in de context van neuro-endocriene aandoeningen, en werpt licht op mogelijke therapeutische wegen die in elke context kunnen worden toegepast.

List of publications

Peer reviewed publications (this thesis)

1. **Correa-da-Silva, F.**, Kalsbeek, M. J., Gadella, F. S., Oppersma, J., Jiang, W., Wolff, S. E. C., Korpel, N. L., Swaab, D. F., Fliers, E., Kalsbeek, A., &; Yi, C.-X. (2023). Reduction of oxytocin-containing neurons and enhanced glymphatic activity in the hypothalamic paraventricular nucleus of patients with type 2 diabetes mellitus. Acta Neuropathologica Communications, 11(1), 107.
2. **Correa-da-Silva, F.**, Fliers, E., Swaab, D. F., &; Yi, C. (2021). Hypothalamic neuropeptides and neurocircuitries in Prader Willi syndrome. Journal of Neuroendocrinology, 33(7).
3. Maya-Monteiro, C. M., **Corrêa-da-Silva, F.**, Hofmann, S. S., Hesselink, M. K. C., la Fleur, S. E., &; Yi, C.-X. (2021). Lipid Droplets Accumulate in the Hypothalamus of Mice and Humans with and without Metabolic Diseases. Neuroendocrinology, 111(3), 263–272.

Accepted publications (peer reviewed)

1. Aguiar, C. F., **Corrêa-da-Silva, F.**, Gonzatti, M. B., Angelim, M. K., Pretti, M. A., Davanzo, G. G., Castelucci, B.G., Monteiro, L. B., Castro, G., Virgilio-da-Silva, J. V., Ribeiro, G., Jaccomo, V., Pereira Andrade, M. C.,Costa, W. L., Gambarini, V., Terra, F. F., Alves-Filho, J. C., Saraiva Câmara, N. O., Boroni, M., … Moraes-Vieira, P. M. (2023). Tissue-specific metabolic profile drives iNKT cell function during obesity and liver injury. Cell Reports, 42(1), 112035.
2. Milanova, I. v., Korpel, N. L., **Correa-da-Silva, F.**, Berends, E., Osman, S., la Fleur, S. E., Fliers, E., Kalsbeek, A., & Yi, C.-X. (2022). Loss of Microglial Insulin Receptor Leads to Sex-Dependent Metabolic Disorders in Obese Mice. International Journal of Molecular Sciences, 23(6), 2933.
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11. Milanova, I. v., **Correa-da-Silva, F.**, Kalsbeek, A., &; Yi, C.-X. (2021). Mapping of Microglial Brain Region, Sex and Age Heterogeneity in Obesity. International Journal of Molecular Sciences, 22(6), 3141.
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Phd portifolio

Name PhD student:	Felipe Correa da Silva
PhD period:	March 2020 – February 2024
Names of PhD supervisors:	
(promotors)	prof. dr. E. Fliers; prof. dr. D.F. Swaab
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General courses, seminars and masterclasses

Animal course (Art. 9) (Utrecht, NL)	2020
ImmunoMetNet AUMC series	2022
Weekly Research meeting Endocrinology and Metabolism	2020–2024
Weekly NIN neuroscience symposium	2020–2024
Designing Translational Health Research Projects: Pitfalls and Opportunities	2023

Conferences

Oral presentations

Society for Neuroscience (Washington, the USA)	2023
Dutch Translational Metabolism Conference (Wageningen, NL)	2023
Annual AG&M PhD retreat (Garderen, NL)	2023
GliaNed (Utrecht, NL)	2023
Foundation for Prader Willi Research Symposium (Chicago, the USA)	2022
Annual AG&M PhD retreat (Garderen, NL)	2022
Annual AG&M PhD retreat (virtual)	2021
Foundation for Prader Willi Research Symposium (virtual)	2020

Poster presentations

16th European Meeting on Glial Cells in Health and Disease	2023
7th Amsterdam Neuroscience Annual Meeting	2022

Attended

EMBO Microglia Workshop (virtual)	2021
Göttingen Meeting of the German Neuroscience Society (virtual)	2021

Teaching

Daniella Verschuur, BSc thesis, medicine (3 months)	2020
Laura Koolman, MSc literature review (3 months)	2020
Jorn Oppersma, BSc internship (6 months)	2020
Erik Kroesbergen, MSc internship (6 months)	2021
Femke S. Gadella, HBO internship (9 months)	2021
Hang Ha, MSc internship (6 months)	2021
Pim Schouten, BSc internship (6 months)	2023
Marten Peters, MSc internship (4 months)	2023

About the author

Felipe Correa da Silva was born on the 03rd of October 1994, in Campinas, southeast of Brazil. After graduation from high school (Anglo Cezanne, Americana) in 2012, a bachelor's degree in Biological Sciences was pursued at the University of Campinas. The choice of becoming a biologist might have come as a surprise to many, but a clear plan was concocted in Felipe's mind. Indeed, to be recognized as a biologist, many challenges were faced – countless hours under the Sun, by the microscope, or trying to understand the anatomy of bugs were spent. It was a Kafkaesque experience, one could romanticize.

In 2017, a combination of context and circumstances led Felipe's scientific interests to Immunology. The topic of choice was the interplay between physiological control of metabolism and immunity, and immunity through the lens of cellular metabolism. A meta-approach to a multilayered question; like a Godard film. Macrophages were the protagonists there, but the urge to understand the brain as a basis for metabolic control was inevitable. This ultimately culminated in moving away to Amsterdam for a brief period to absorb new knowledge. This brief stay unfolded into the opportunity to become a PhD student there, with the aim of understanding the role of brain immune cells in metabolic diseases. A MSc in Molecular Biology (Immunology) was achieved in 2019.

Felipe moved for the second time to Amsterdam days before the first COVID-19 lockdown in March 2020. With adjustments, the proposed research was developed, and it is mostly explicit in this thesis. Immense support was received throughout these years. The PhD experience provided the opportunity of mentoring, to deepen into neuroimmunology and to (try to) craft meaning out of small pieces of brain. Maybe it is still too soon to create a parallel to any piece of art for the whole experience.

A cinematic guide to the author:

- *Persona* (Ingmar Bergman, 1966);
- *Daises* (Věra Chytilová, 1966);
- *La Chinoise* (Jean-Luc Godard, 1967);
- *Crimes of the Future* (David Cronenberg, 1970);
- *Pink Flamingos* (John Waters, 1972);
- *Bubble bath* (György Kovásznai, 1980);
- *Charlie and the Chocolate Factory* (Tim Burton, 2005);
- *I'm a cyborg, but that is OK* (Park Chan-wook, 2006);
- *Synecdoche, New York* (Charlie Kaufman, 2009);
- *Midsommar* (Ari Aster, 2019).

Acknowledgements

I feel that this PhD had so much of me that sometimes it was hard to even dissociate the self from what I was doing on the benches or microscope. This thesis can be seen as a thinly veiled ideology that was built upon over and over. So many changes I have been experiencing and somewhat influenced not just me, but how this PhD unfolded. At times, my experience in Amsterdam was like **Paris, Texas** (Wim Wenders, 1984) – fragmented but shot in beautiful scenery; and science made some of these fragments coherent. This writing process felt like I was staring at **Naked Lunch** (David Cronenberg, 1991), with some elements more present than others. Because science is a collective matter and it can only be built collectively, this section is a celebration of all the help I have had in all those years. However, I must confess that I am at a loss for words and I will try my best to convey my gratitude. I thought it was very fitting to make cinematic guides of the content of this thesis, scattered in different places, including this section.

First, I would like to leave my appreciation and deep gratitude to all persons, and their loved ones, who donated their brains so the research I conducted was possible. My only hope is that I could have crafted positive meaning out of those brain pieces, other than the clinical or pathological one. I wish I could personally recommend **Coda** (Alan Holly, 2015) to you.

Dr. Chun-Xia Yi, dear **Chun-Xia**, thank you for your mentorship and support in the last years! Your dedication and devotion to science were, and are, a model to pursue. Your blunt honesty, sharp eyes, unconventional thinking and passion for microglia were there every time I needed them. I leave your lab having grown knowledge, and I hope to have met your expectations. Our communication was never long winded, so I will limit it to say that I feel honored to have been your student. I wish you success with the Yi dynasty! My movie recommendation to you is **The Royal Tenenbaums** (Wes Anderson, 2001). I think you will like the symmetry, the plot and how simplicity is what actually composes all the chaos.

Professor dr. Eric Fliers, dear **Eric**, my deepest gratitude for your mentorship and immense help in all those years. I also feel very honored to have been mentored by you. Your calm, kindness and wisdom were fundamental for my trajectory in the Netherlands. Your perspective often brought a different meaning to my work, and a nice reminder of the societal importance of what was being done. My movie recommendation to you is **The cook, the thief, his wife, her lover** (Peter Greenaway, 1989) – I think you will appreciate the imagery and all the symbolism in this movie.

Professor dr. Dick Swaab, dear **Dick**, thank you for your mentorship and valuable inputs to my work. I really appreciate all the moments I spent in your lab, where I learned so much about the human brain and all about the hypothalamus intricacies with you. The work in this thesis could not have been done without your help and dedication to human brain studies throughout the years. It was a privilege learning from you. My movie recommendation to you is **A Hora da Estrela** (Hour of the star, Suzana Amaral, 1985). I believe you will like how the plot unfolds.

Prof. dr. Andries Kalsbeek, dear **Dries**, I am extremely grateful for all the support and knowledge I got from you during the past years. Your calmness and very reasonable words were fundamental to my progression. Your devotion to understanding chronobiology and the SCN is an inspiration to me! I hope I made justice to your mentorship. My movie recommendation to you is **Anatomy of time** (Jakrawal Nilthamrong, 2021). I think you will appreciate how time is a subject by itself here.

To my **defence committee**, thank you for taking the time to help me to be a better scientist. To you, as a group, I recommend **Living in Oblivion** (Tom DiCillo, 1995).

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also important during my period in Brazil. It is hard to think of a film that all of you could appreciate, my best guess is **Todo Sobre Mi Madre** (Pedro Almodovar, 1999).

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To all (former) members of the Yi group, with whom I had the opportunity to learn from. **Martin**, who supervised me through my internship during my Masters and paved the way of human studies in the Yi group. My recommendation to you is **Adaptation** (Spike Jonze, 2003). **Nikita**, who has always been kind and incredibly helped me though the beginning of the PhD journey. **Cinema Paradiso** (Giuseppe Tornatore, 1990) reminds me of you. **Samantha**, thank you for always checking up on me and for the interesting talks we had, they were not often enough but certainly fun to have; **You, the living** (Roy Anderson, 2007) is a movie I think you will enjoy. **Clarissa**, not exactly a Yi group member, but we have closely worked together for years now. I do not have the words, in all honesty. I do not believe I could have done it without your help. Your limitless passion to research, your strong moral compass and kindness inspired me all the way through – **Valerie and Her Week of Wanders** (Jaromil Jires, 1970) is a fantastic surreal tale to be seen. **Fernando**, what a joy the time we spent together! I really miss you

here in Amsterdam. Your scientific eagerness and determination were, and are still, an inspiration. I think you will like **Liquid Sky** (Slava Tsukerman, 1982). **Han**, thank you for the countless support and knowledge that you shared since you arrived - **Three Times** (Hou Hsiao-hsien, 2005) might be a movie that you enjoy. **Wei**, thank you for all the opportunities to discuss science and the help during the time you were in the lab – **Shanghai Triad** (Zhang Yimou, 1995) is a film I think you will like. **Jarne**, best of luck in your PhD journey; my movie recommendation to you is **Cube** (Vincenzo Natali, 1997). **Shanshan**, my gratitude for all the knowledge shared with us! To you, I suggest watching **Spring in a Small Town** (Mu Fei, 1948). **Xiaolan**, my appreciation for your dedication to your (challenging) projects – my film recommendation to you is **Children Who Chase Lost Voices** (Makoto Shinkai 2011). To all the students that I had contact or responsibility to mentor in this period – **Daniella**, **Laura**, **Jorn**, **Erik**, **Femke**, **Hang**, **Pim**, **Marten**, **Athina**, **Jenny**, **Milan**, **Sanne**. Thank you for the opportunities and the inputs to be a better mentor. To find a collective movie recommendation here was challenging, but I think **My Dinner with Andre** (Louis Malle, 1981) can be an interesting watch.

To the Endo department staff. Prof. dr. Susanne la Fluer, dear **Susanne**, thank you for the support, patience, motivation and guidance that you kindly offered me along those years. My movie recommendation to you is **But I'm A Cheerleader** (Jamie Babbit, 2000). Prof.dr. Anita Boelen, dear **Anita**, thank you for your support and thoughtful comments along these years – I was unsure what to recommend here as well, but my guts tell me you might like **Cold war** (Pawel Pawlikowski, 2018). **Cecilia**, I cannot appreciate enough your caring words and how welcomed you made me feel here. *Te lo agradezo de todo corazón!* **76 89 03** (Cristian Bernard and Flavio Nardini, 2000), is a movie I think you might appreciate. **Olga**, darling, thank you for cheering up my days, and our ever-evolving discussions on life (goals). **The Draughtsman's Contract** (Peter Greenaway, 1982) can be of your interest. **Unga**, thank you for your impeccable care of the lab. We have not overlapped much in time, but most of the work with the PWS material is somehow a continuation of your own studies! **Perfect Days** (Wim Wenders, 2023) can be of your taste I believe. **Ewout** and **Daniela**, thank you for your motivational words, inputs and solicitude, always there since the internship period - I think you both will like **Dr. Strangelove or How I Learned to Stop Worrying and Love the Bomb** (Stanley Kubrick, 1964). **Tess** and **Kim**, I appreciate the nice talks we had and all the efforts you

have made to make life at the lab easier! **Loose Cannons** (Ferzan Özpetek, 1997) and **Vertigo** (Alfred Hitchcock, 1958) are films I believe you will enjoy, respectively. To the Kalsbeek group, of which people were always there for a cup of coffee, vivid scientific discussions or just to hear my complaints about life. **Ayano** (**Zuckerbaby**, Percy Adlon, 1985), **Anayancy** (**The Double Life of Véronique**, Krzysztof Kieślowski 1995), **Delaram** (**The boy friend**, Ken Russel 1971), **Valentina** (**Soul Kitchen**, Faith Akin 2009), **Paul** (**The Rendez-Vous of Déjà-vu**, Antonin Peretjako 2013) and **Wayne** (**The Darjeeling Limited**, Wes Anderson 2007).

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My deepest apologies if someone was forgotten. I tried my best to convey my gratitude and to remember everyone.

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To whoever can solve the riddle: "C'est une pipe?", please share the answer!

' ...I have become a foreigner in a foreign land.'
Exodus 2:22