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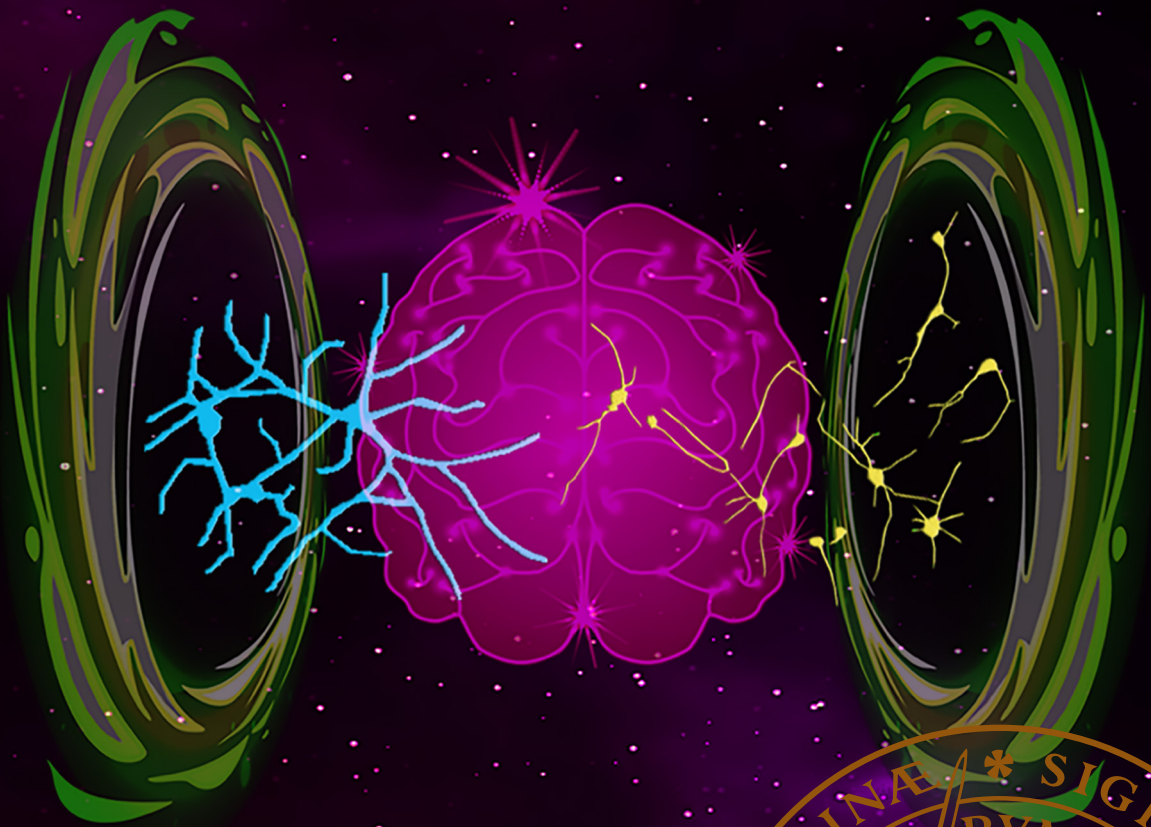
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Generation of Human Subtype-Specific Interneurons for Therapeutic Replacement and Disease Modelling

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EXPERIMENTAL MEDICAL SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY





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Christina-Anastasia Stamouli



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UNIVERSITY

DOCTORAL DISSERTATION

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Abstract:

GABAergic interneurons compose the main inhibitory neurons in the cerebral cortex and medial ganglionic eminence (MGE)-derived interneurons, including parvalbumin (PV) and somatostatin (SST) interneurons, account for approximately 70% of cortical interneurons. PV and SST interneurons differ in their morphology, functionality and connectivity, resulting in distinct inhibitory modes within circuits. Their loss or dysfunction has been involved in the pathology of several neurodevelopmental disorders, such as schizophrenia, epilepsy and autism spectrum disorder. Yet, the exact pathology of PV and SST is unknown. Stem cell and reprogramming approaches allow for derivation of human subtype-specific interneurons *in vitro*. These can be used for disease modelling when derived from patient cells e.g. via iPSCs or as a source for cell replacement when transplanted to the brain. Direct reprogramming, where a somatic cell, e.g. glia converts to a neuron, has been proposed as a cell replacement strategy, offering a fast approach for neuron derivation that can be applied *in situ*.

In this thesis, the overall aim was to develop methods to derive PV and SST interneurons for future therapeutic cell replacement strategies and disease modelling. Firstly, we demonstrated for the first time that human PV interneurons can be derived from reprogrammed glia *in vitro*. Using single-cell sequencing, we unravelled their lineage trajectory, showing that they were of *bona fide* equivalence. This study showed proof-of-principle for human interneuron reprogramming that in the future can be applied *in vivo* targeting resident glia. In paper II we transplanted reprogrammed neurons into the mouse prefrontal cortex (PFC). We demonstrated that human reprogrammed neurons could survive up to 10 months and integrate into the host circuitry, functionally and structurally maturing over time. In paper III, we successfully derived human SST interneurons from embryonic stem cells (ESCs) in a 3D co-culture system and unravelled their subclass characteristics. This protocol was then applied to schizophrenic patient-derived iPSCs and showed that patient-derived interneurons are functionally impaired, and they undergo an extensive GABAergic signalling remodelling.

Altogether, these findings provide evidence for successful derivation of human subtype-specific interneurons and lay a solid foundation for their application for therapeutic cell replacement and disease modelling.

Key words: interneurons, PV, SST, direct reprogramming, neuronal differentiation, human glia, stem cells, cell replacement therapy, disease modelling

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Christina-Anastasia Stamouli



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To everyone who stood by me when it mattered most

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Abstract

GABAergic interneurons compose the main inhibitory neurons in the cerebral cortex and medial ganglionic eminence (MGE)-derived interneurons, including parvalbumin (PV) and somatostatin (SST) interneurons, account for approximately 70% of cortical interneurons. PV and SST interneurons differ in their morphology, functionality and connectivity, resulting in distinct inhibitory modes within circuits. Their loss or dysfunction has been involved in the pathology of several neurodevelopmental disorders, such as schizophrenia, epilepsy and autism spectrum disorder. Yet, the exact pathology of PV and SST is unknown. Stem cell and reprogramming approaches allow for derivation of human subtype-specific interneurons *in vitro*. These can be used for disease modelling when derived from patient cells e.g. via iPSCs or as a source for cell replacement when transplanted to the brain. Direct reprogramming, where a somatic cell, e.g. glia converts to a neuron, has been proposed as a cell replacement strategy, offering a fast approach for neuron derivation that can be applied *in situ*.

In this thesis, the overall aim was to develop methods to derive PV and SST interneurons for future therapeutic cell replacement strategies and disease modelling. Firstly, we demonstrated for the first time that human PV interneurons can be derived from reprogrammed glia *in vitro*. Using single-cell sequencing, we unravelled their lineage trajectory, showing that they were of *bona fide* equivalence. This study showed proof-of-principle for human interneuron reprogramming that in the future can be applied *in vivo* targeting resident glia. In paper II we transplanted reprogrammed neurons into the mouse prefrontal cortex (PFC). We demonstrated that human reprogrammed neurons could survive up to 10 months and integrate into the host circuitry, functionally and structurally maturing over time. In paper III, we successfully derived human SST interneurons from embryonic stem cells (ESCs) in a 3D co-culture system and unravelled their subclass characteristics. This protocol was then applied to schizophrenic patient-derived iPSCs and showed that patient-derived interneurons are functionally impaired, and they undergo an extensive GABAergic signalling remodelling.

Altogether, these findings provide evidence for successful derivation of human subtype-specific interneurons and lay a solid foundation for their application for therapeutic cell replacement and disease modelling.

Lay summary

The brain, one of the most complex and fascinating organs of the human body, is regulating functions ranging from basic processes, such as body temperature, to decision making. The brain is mainly made up from cells called neurons and there are different types. One important type is GABAergic interneurons. These cells are essentially responsible to putting a brake in excessive brain activity. In some disorders, such as epilepsy, schizophrenia and autism spectrum disorder, these important neurons are either lost or they don't function properly, leading to problems in brain activity.

To study interneurons in these diseases, researchers have developed different advanced strategies to derive them in the lab. One of them is a called direct reprogramming, a process that forces a cell, such as a supporting glia cell, to switch its fate and convert to another cell, in this case an interneuron, by delivering specific neuronal genes. The main advantage of this strategy is its potential application directly in the brain to replace the lost cells. One other breakthrough strategy is the generation of induced pluripotent stem cells (iPSCs). In this process, a body cell, such as a skin cell, is going back to a stem cell state and from there scientists can mimic normal development, in a process called differentiation, to generate interneurons. With this technology, skin cells from patients can be converted into stem cells to make interneurons. These interneurons will carry the disease and researchers will attempt to find the mechanisms behind it and identify new treatment targets.

In this thesis, we have derived human interneurons in the lab with direct reprogramming or with differentiation strategies from iPSCs. In the first study, we reprogrammed human glial cells that exist in the brain and support neurons' function into interneurons. Importantly, we proved that these interneurons are the same as the ones normally found in the human brain. We also transplanted these interneurons into a mouse brain and proved that they could survive into a brain environment. Furthermore, we created a model for differentiation of stem cells into interneurons, which exhibited characteristics of interneurons found in a healthy brain. Then we used this model to induced pluripotent stem cells coming from schizophrenic patients to differentiate them into interneurons and we found a damage in their electrical function and protein expression, potentially contributing to the disease.

In summary, this thesis has investigated and demonstrated that it is possible to generate human interneurons in the lab using advanced approaches. These can in the future be used for cell replacement and for studying disease mechanism in the lab. This an important step toward generating interneurons for therapeutic replacement and investigation of disease mechanisms, bringing these approaches closer to clinic.

Populärvetenskaplig sammanfattning

Hjärnan, ett av de mest komplexa organen i människokroppen, den reglerar funktioner som sträcker sig från grundläggande processer, såsom kroppstemperatur, till beslutsfattande. Hjärnan består av celler som kallas neuroner och det finns olika typer. En viktig typ är GABAerga interneuroner. Dessa celler är ansvariga för att bromsa överdriven hjärnaktivitet. Vid vissa sjukdomar, såsom epilepsi, schizofreni och autismspektrumstörning, om dessa viktiga neuroner är förlorade eller om de inte fungerar korrekt, leder det till problem i hjärnaktiviteten.

För att undersöka interneuroner har forskare utvecklat olika avancerade strategier för att återskapa dem i labbet. En av dem är så kallad direkt omprogrammering, där man tvingar en cell, t.ex. en stödjecell att ändra sitt öde genom att uttrycka specifika nervcellsgener så de omvandlas till en annan cell, såsom en interneuron cell. Den största fördelen med denna strategi är att man i framtiden skulle kunna applicera den direkt i hjärnan genom att omvandla befintliga stödjeceller till interneuron. En annan banbrytande strategi är återskapa mänskliga interneuron via inducerade pluripotenta stamceller (iPSCs). I denna process återgår en kroppscell, såsom en hudcell, till ett stamcellstillstånd och därifrån kan forskare skapa en interneuron cell, i en process som kallas differentiering. Med denna teknik kan hudceller från patienter återgå till stamceller för att skapa interneuroner i labbet. Dessa interneuroner kommer att bära på sjukdomsgenerna, och kan därför likna de riktiga interneuronen i patientens hjärna. Man kan titta på sjukdomsmekanismen i labbet och försöka hitta nya behandlingar med denna teknik.

I denna avhandling undersöker vi mänskliga interneuron framtagna i labbet med direkt omprogrammering eller iPSCs cellsstrategier. Vi bevisade att omprogrammerade interneuroner från stödjeceller är desamma som de som normalt finns i den mänskliga hjärnan. Vi transplanterade också dessa nervceller till en mushjärna och bevisade att de kunde överleva i en levande hjärnan, vilket stödjer en fortsatt utveckling för cellterapi. Dessutom har vi utvecklat mänskliga interneuron från stamceller, som uppvisade egenskaper av en annan celltyp, också sjukdomsrelevanta. Dessa togs sedan fram för iPSCs från schizofrena patienter och vi fann att patientcellerna var skadade i sin elektriska funktion och proteinuppsättning.

Sammanfattningsvis har denna avhandling undersökt och visat att det är möjligt att generera mänskliga interneuroner i laboratoriet med hjälp av avancerade metoder.

Dessa kan i framtiden användas för cellersättning och för att undersöka sjukdomsmekanismer i laboratoriet. Detta är ett viktigt steg mot att utveckla interneuroner för terapeutisk ersättning och undersökning av sjukdomsmekanismer, vilket för dessa metoder närmare kliniken.

Περίληψη στα ελληνικά

Ο εγκέφαλος, ένα από τα πιο πολύπλοκα και μαγευτικά όργανα του ανθρώπινου σώματος, ρυθμίζει λειτουργίες που κυμαίνονται από βασικές διαδικασίες, όπως η ρύθμιση της θερμοκρασίας του σώματος, έως την λήψη αποφάσεων. Ο εγκέφαλος αποτελείται κυρίως από κύτταρα που λέγονται νευρώνες και υπάρχουν διαφορετικοί τύποι νευρώνων. Ένας από αυτούς είναι οι GABAεργικοί ενδιάμεσοι νευρώνες. Αυτά τα κύτταρα είναι υπεύθυνα για να βάζουν φρένο στην υπέρμετρη λειτουργία του εγκέφαλου. Σε κάποιες ασθένειες, όπως η επιληψία, η σχιζοφρένεια και η διαταραχή φάσματος του αυτισμού, αυτοί οι σημαντικοί νευρώνες είτε χάνονται, είτε δε λειτουργούν σωστά. Αυτό έχει ως αποτέλεσμα τα προβλήματα στην λειτουργία του εγκέφαλου.

Για να μελετηθούν οι ενδιάμεσοι νευρώνες σε αυτές τις ασθένειες, οι επιστήμονες έχουν αναπτύξει διαφορετικές προηγμένες μεθόδους για να τους παράγουν στο εργαστήριο. Μια από αυτές λέγεται απευθείας επαναπρογραμματισμός, μια διαδικασία που αναγκάζει ένα κύτταρο, όπως ένα υποστηρικτικό γλοιακό κύτταρο, να αλλάξει την ταυτότητά του και να μετατραπεί σε ένα άλλο κύτταρο, σε αυτήν την περίπτωση έναν ενδιάμεσο νευρώνα, παρέχοντας συγκεκριμένα νευρικά γονίδια. Το κύριο πλεονέκτημα αυτής της στρατηγικής είναι η πιθανή εφαρμογή της μετατροπής απευθείας στον εγκέφαλο, για την αντικατάσταση των χαμένων κυττάρων. Μια άλλη καινοτόμος στρατηγική είναι η παραγωγή επαγόμενων βλαστικών κυττάρων. Σε αυτή την διαδικασία, ένα σωματικό κύτταρο, π.χ. ένα κύτταρο δέρματος, επιστρέφει σε κατάσταση βλαστικού κυττάρου και μετά οι επιστήμονες μιμούνται την φυσιολογική ανάπτυξη, με μια διαδικασία που ονομάζεται διαφοροποίηση, για να δημιουργήσουν ενδιάμεσους νευρώνες. Με αυτή την τεχνολογία, δερματικά κύτταρα από ασθενείς μπορούν να μετατραπούν σε βλαστικά κύτταρα για την παραγωγή ενδιάμεσων νευρώνων. Αυτοί οι ενδιάμεσοι νευρώνες θα φέρουν την ασθένεια και οι ερευνητές θα προσπαθήσουν να βρουν τους μηχανισμούς που κρύβονται από πίσω και να εντοπίσουν νέους θεραπευτικούς στόχους.

Σε αυτή την διατριβή, έχουμε δημιουργήσει ενδιάμεσους νευρώνες στο εργαστήριο με απευθείας επαναπρογραμματισμό ή από διαφοροποίηση επαγόμενων βλαστικών κυττάρων. Στην πρώτη μελέτη, επαναπρογραμματίσαμε ανθρώπινα γλοιακά κύτταρα, τα οποία υπάρχουν στον εγκέφαλο και στηρίζουν την λειτουργία των νευρώνων, σε ενδιάμεσους νευρώνες. Αποδείξαμε ότι οι συγκεκριμένοι ενδιάμεσοι νευρώνες είναι παρόμοιοι με αυτούς που υπάρχουν φυσιολογικά στον ανθρώπινο

εγκέφαλο. Επίσης, μεταμοσχεύσαμε αυτούς τους ενδιάμεσους νευρώνες σε εγκέφαλο ποντικού και αποδείξαμε ότι μπορούν να επιβιώσουν στο περιβάλλον του εγκέφαλου. Επιπρόσθετα, δημιουργήσαμε ένα μοντέλο για διαφοροποίηση βλαστικών κυττάρων σε ενδιάμεσους νευρώνες, οι οποίοι έχουν τα ίδια χαρακτηριστικά με αυτούς που υπάρχουν σε έναν υγιή εγκέφαλο. Στη συνέχεια, χρησιμοποιήσαμε αυτό το μοντέλο σε επαγόμενα βλαστικά κύτταρα από ασθενείς με σχιζοφρένια για τα διαφοροποιήσουμε σε ενδιάμεσους νευρώνες. Εντοπίσαμε μια βλάβη στην ηλεκτρική τους λειτουργία και στην έκφραση πρωτεϊνών, η οποία ενδεχομένως συμβάλλει στην εκδήλωση της ασθένειας.

Εν κατακλείδι, σε αυτή την διατριβή ερευνήσαμε και αποδείξαμε ότι είναι δυνατή η δημιουργία ανθρωπίνων ενδιάμεσων νευρώνων στο εργαστήριο με προηγμένες τεχνικές. Αυτοί μπορούν στο μέλλον να χρησιμοποιηθούν για την αντικατάσταση των χαμένων κυττάρων και για τη μελέτη μηχανισμών παθογένειας. Αυτό αποτελεί ένα σημαντικό βήμα προς την δημιουργία ενδιάμεσων νευρώνων για θεραπευτική αντικατάσταση και διερεύνηση μηχανισμών ασθενειών, φέρνοντας αυτές τις προσεγγίσεις πιο κοντά στην κλινική εφαρμογή.

Original Articles

Paper I

A distinct lineage pathway drives parvalbumin chandelier cell fate in human interneuron reprogramming.

Stamouli, C. A., Degener, A., Cepeda-Prado, E., Bruzelius, A., Andersson, E., Giacomoni, J., Vorgeat, A. D., Kidnapillai, S., Klementieva, O., Parmar, M., Olariu, V., & Rylander Ottosson, D.

Sci Adv, 12(1), eadv0588, 2026

Paper II

Long-Lasting structural and functional maturation in Transplanted Human neurons reprogrammed from Glial progenitors

Cepeda-Prado, E., **Stamouli, C. A.**, Nocera, G., Aretio-Medina, C., Kidnapillai, S., Bruzelius, A., Giacomoni, J., Kajtez, J., Parmar, M., Rylander Ottosson, D.

Submitted manuscript 2026

Paper III

Three-dimensional co-culturing reveals human stem cell-derived somatostatin interneurons with subclass expression

Bruzelius, A., **Stamouli, C. A.**, Holldobler, A. L., Aretio-Medina, C., Cepeda-Prado, E., Sozzi, E., Passarello, G. R., Nocera, G., Giacomoni, J., Olariu, V., & Rylander Ottosson, D.

Stem Cell Reports, 20(9), 102634, 2025

Paper IV

hiPSC-derived interneurons from schizophrenia parent-case trio reveal GABAergic signalling alterations.

Stamouli, C. A., Piochon, C., Cepeda-Prado, E., Apati, A., Réthelyi, J., Rylander Ottosson D.

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Abbreviations

2D	2-dimensional
3D	3-dimensional
ABM	<i>Ascl1, Brn2, Myt1l</i>
ALN	<i>Ascl1, Lmx1a, Nurr1</i>
ADLSF	<i>Ascl1, DLX5, LHX6, Sox2, FOXG1</i>
AP	Action Potential
BDNF	Brain-derived Growth Factor
CGE	Caudal Ganglionic Eminence
DIV	Days <i>in vitro</i>
DPI	Days post-injection
EB	Embryoid bodies
GABA	γ -Aminobutyric Acid
GE	Ganglionic Eminence
GFP	Green Fluorescent Protein
GM	Glial Medium
GSEA	Gene Set Enrichment Analysis
hGPCs	human Glial Progenitor Cells
hIN	human interneurons
hiN	human induced Interneurons
iPSCs	induced pluripotent stem cells
Lam	Laminin
LGE	Lateral Ganglionic Eminence
mAST	mouse Astrocytes
MGE	Medial Ganglionic Eminence
MEF	Mouse Embryonic Fibroblasts
MOI	Multiplicity of Infection
MPT	Months post-transplantation
MZ	Marginal Zone

ND	Neuronal medium
NPCs	Neuronal Precursor Cells
OPCs	Oligodendrocyte progenitor cells
PBS	Phosphate buffer solution
PFA	Paraformaldehyde
PLO	Polyornithine
PN	Principal Neuron
PTX	Picrotoxin
PV	Parvalbumin
RT-qPCR	Real-time quantitative Polymerase Chain Reaction
SCZ	Schizophrenia
Shh	Sonic hedgehog
snRNA-seq	Single-nuclei RNA sequencing
SST	Somatostatin
SVZ	Subventricular Zone
TetO	Tet On
TF	Transcription factor
ULA	Ultra-low attachment
UMAP	Uniform Manifold Approximation and Projection

Introduction

Interneurons

Role of interneurons in the cerebral cortex

Human cerebral cortex, one of the most important and complicated structures, plays crucial role in processing sensory information, controlling motor output, and mediating higher-order cognitive functions, such as decision-making (Carlen, 2017). This complicated brain circuitry consists of two main neuronal types: excitatory projection neurons (PNs) and inhibitory GABAergic interneurons (INs) (Lim et al., 2018; Marin, 2012; Tasic et al., 2016; Tremblay et al., 2016).

GABAergic INs are critical for the proper function of cerebral cortex. The typical idea is that interneurons are responsible for network coordination, where their firing is correlated to their afferent connections. Additionally, they can be responsible for the control of the flow of information and are excited at precise moments in response to specific stimuli. The impact of INs in their local network relies on the connectivity to the postsynaptic neuron, which is dependent on their developmental programme (Kepecs & Fishell, 2014).

Inhibitory INs have been considered to simply protect networks against excessive excitation (Douglas et al., 1995). In recent years, our understanding of their function has significantly advanced. Studies have shown that they could normalize the activity of local excitatory networks and provide feed-forward inhibition. Other functions include cross-coupling, lateral inhibition for PN populations' separation, and disinhibition for increase of local activity (Kepecs & Fishell, 2014). Inhibition is not only crucial for activity balance, but also provides a functional diversity in the network dynamics, increasing the computational power of cortical circuits (Buzsaki & Draguhn, 2004; X. J. Wang et al., 2004).

Origin, subtype specification and maturation of interneurons

Cortical GABAergic INs originate from a transitory brain region in the ventral telencephalon called the subpallium and from there they migrate to their destination in the cortex (Anderson et al., 1997). Subpallium in rodents and humans can be divided into separate zones; caudal ganglionic eminence (CGE), lateral ganglionic

eminence (LGE), medial ganglionic eminence (MGE), preoptic area (POA) and adjacent preoptic-hypothalamic (POH) border domain and the septum. Cortical INs derive mainly from MGE, CGE and few from POA (Lim et al., 2018). Research in the last years utilising foetal human tissue has demonstrated that a portion of cortical interneurons derives from dorsal telencephalon, something that has not been observed in mice (Marin, 2025).

Several key transcription factors (TFs) are highly expressed in the neural precursor cells (NPCs) the of the ventral telencephalon, forming regulatory networks and controlling interneuron development. These regulatory networks seem to be highly conserved between rodents and humans (Marin, 2025). *Dlx1* and *Dlx2* genes ensure interneuron fate (Cobos et al., 2005; Stuhmer et al., 2002). *Ascl1* is a key transcription factor in IN specification and its loss leads to reduced number of NPCs in the MGE and reduced number of interneurons in the cortex (Casarosa et al., 1999). *Dlx5* and *Dlx6* are expressed in both MGE and CGE and function downstream of *Dlx1/2* and *Ascl1* (Eisenstat et al., 1999).

Each subpallial domain is characterized by the expression of different transcription factors that give rise to different interneuron subtypes. MGE-derived interneurons account for approximately 70% of cortical interneurons and give rise to two main categories of subtype-specific interneurons: parvalbumin (PV) and somatostatin (SST) (Sultan et al., 2013). Interestingly, single-cell sequencing studies in human samples have identified an interneuron population that co-expresses LAMP5 and LHX6, which is rare in rodents (Krienen et al., 2020). *Nkx2.1* is primarily expressed in MGE (Sussel et al., 1999). In *Nkx2.1* null mutant mice, nearly all PV and SST interneurons are absent (Butt et al., 2008). *Nkx2.1* expression is maintained by Sonic hedgehog (Shh) signalling. High Shh signalling activity promotes of SST fate at the expense of PV interneurons (Xu et al., 2010). *Lhx6* functions downstream of *Nkx2.1* to drive the differentiation of both PV and SST interneurons (Du et al., 2008; Liodis et al., 2007). It also regulates the migration and laminar position of INs (Vogt et al., 2014). Transcription factors *Maf* and *Mafb*, expressed in the subventricular zone (SVZ) of MGE, promote PV fate at the expense of SST interneurons (Pai et al., 2020; Pai et al., 2019).

It is poorly understood how interneuron diversity is generated within each domain. Lineage tracing and transplantation studies have demonstrated that dorsal MGE is biased towards SST interneurons, while ventral MGE mostly produced PV neurons (Flames et al., 2007; Wonders et al., 2008). Clonal studies have shown that individual progenitor cells in MGE can generate both PV and SST interneurons (Ciceri et al., 2013). This suggest that intrinsic molecular mechanisms are not enough to explain interneuron specification, with the microenvironment contributing to this process. A recent study has shown that spatial dynamics play a crucial role in interneuron specification, suggesting their fate acquisition is malleable (Mostajo-Radji et al., 2025). Temporal dynamics also influence

interneuron specification. SST interneurons are born earlier, while PV interneurons are generated throughout neurogenesis (Miyoshi et al., 2007).

INs reach cortex through tangential migration and occupy all cortical layers (Figure 1). Several signalling pathways and molecules are involved in this migratory process. For example, BDNF drives the tangential migration of MGE-derived INs through TrkB signalling (Polleux et al., 2002). Additionally, the expression dynamics of HGF in the ventral telencephalon reflect interneuron migration patterns (Powell et al., 2001). GDNF family receptor alpha-1 (GFR α 1) is co-expressed with Lhx6 in the MGE mantle zone (Pozas & Ibanez, 2005). Many chemorepellents and chemoattractants influence interneuron migration. Chemorepellents are primarily expressed in the subpallium to push newborn INs away from this region (Wichterle et al., 2003). Cortical interneurons expressing neuropilin are repelled by striatal cells expressing semaphorins (Marin et al., 2001). Moreover, Robo1 regulates cortical interneuron migration via semaphorins (Marin et al., 2001). On the other hand, chemoattractants are primarily expressed in the pallium to direct interneurons to the neocortex. A typical chemo attractive factor is neuregulin-1 (NRG1). In the telencephalon at E13.5, diffusible NRG1 is expressed in the cortex (Flames et al., 2004). It is worth noting that in humans GABAergic INs continue to migrate to the prefrontal cortex (PFC) for several months after birth (Marin, 2025).

Various studies have shown that IN laminar distribution in the cortex depends on several factors, such as cell-cell interaction, intrinsic programmes and neuronal activity (Figure 1). The final laminar distribution of INs is regulated by Nrg3, secreted by PNs, and its interaction with the receptor ErbB4 expressed on INs (Bartolini et al., 2017). MGE-derived INs follow an inside-out lamination pattern, where early born neurons populate deep layers, while later-born neurons more superficial layers (Angevine & Sidman, 1961). Furthermore, PNs' cell identity impacts IN distribution in the cortex. For example, ectopic expression of Fezf2, an important transcription factor for corticofugal neurons, in intratelencephalic neurons changes the laminar distribution of PV and SST INs (Ye et al., 2015). Neuronal activity marks the end of distribution period, with GABA switch playing a vital role in this process. While GABA is depolarizing in newborn INs, it switches to hyperpolarizing once the INs reach their final destination. This switch is mediated by the increased expression of the potassium/chloride exchanger KCC2, which allows chloride ions' flow into the neurons (Bortone & Polleux, 2009).

The brain produces an excess of INs during development, to ensure that enough INs integrate into the cortical networks, discarding the surplus neurons once the network is formed. The number of both PNs and INs is reduced during the first two postnatal weeks in mice, where around 30%-40% of all INs generated during neurogenesis undergo developmental cell death (Southwell et al., 2012). It has been shown that INs with strong input from glutamatergic neurons have increased chances of survival. Studies manipulating glutamatergic neurons' activity during this critical period demonstrate deviated number of INs (Wong et al., 2018).

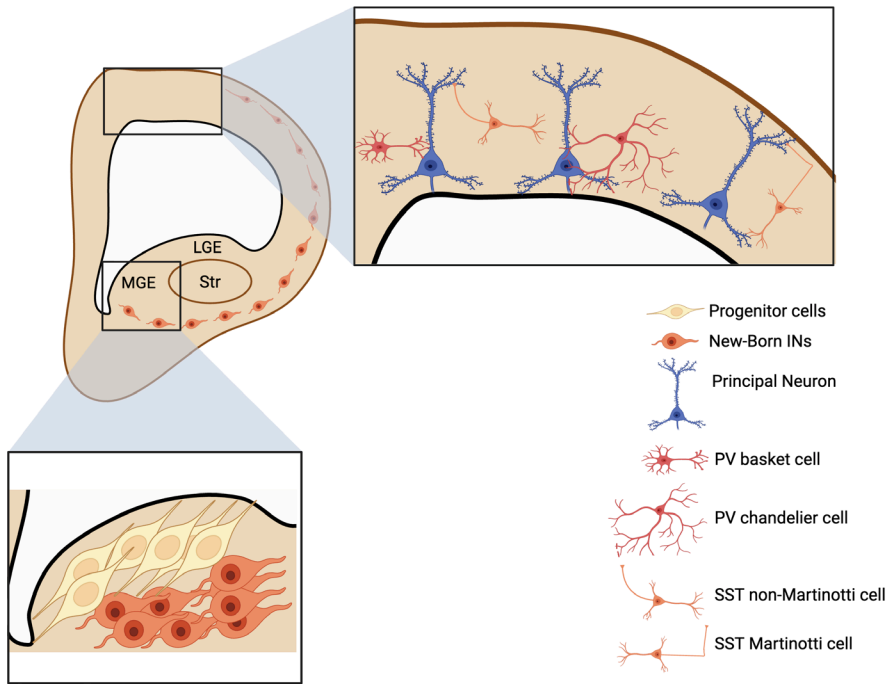


Figure 1. Illustration showing MGE-derived interneuron genesis, migration and laminar distribution

MGE= Medial ganglionic eminence, LGE=Lateral ganglionic eminence, Str=Striatum. Illustration created with Biorender.

PV and SST interneurons

PV interneurons are the largest group of cortical interneurons, and they can be further subdivided into two main categories: basket and chandelier. They have unique electrophysiological characteristics, defined by high energy expenditure, including fast action potential (Llorca & Deogracias, 2022). Chandelier cells have the most typical interneuron morphology with elaborated axons (Inda et al., 2009) and they form synapses onto the axon initial segment of PNs (Taniguchi et al., 2013). They mainly populate layers 2 and 6 and have heterogenous regional distribution across cortical regions (Inda et al., 2009). Most of PV interneurons belong to basket subclass, which exhibit multiple axons and form synapses onto the soma or the proximal dendrites of PNs, driving strong inhibition. They are primarily located from layer 2 to 6 and are widespread in all cortical areas (Hu et al., 2014). Recent single-cell sequencing studies on mouse and human brains have identified more subclasses for PV interneurons, with basket cells showing the most diversity (Bakken et al., 2018; Hodge et al., 2019; Tasic et al., 2016; Tasic et al., 2018). Most PV basket cells are enclosed by a special extracellular matrix called perineuronal

net. This structure regulates the contribution of PV+ interneurons to developmental critical periods and synaptic plasticity (Fawcett et al., 2019).

Both intrinsic and extrinsic factors play a role in PV maturation and synapse formation. Overexpression of the enzyme GAD67, responsible for GABA production, enhances the maturation of the perisomatic connections (Chattopadhyaya et al., 2007). Moreover, network activity dynamically modulates PV IN properties via the transcriptional regulator Er81 (Dehorter et al., 2015). A recent study showed that maturation of PV interneurons is triggered by neuronal activity and mediated by PGC-1 α (Moissidis et al., 2025). Signalling cues, such as tonic activation of the NMDA glutamate receptors regulates maturation of PV interneurons (Hanson et al., 2019; Pan-Vazquez et al., 2020). Lgi2 regulates the formation of basket cells perisomatic synapses, while Fgf13 regulates axo-axonic synapses onto the axon initial segment of PNs (Favuzzi et al., 2019).

SST interneurons are the second largest interneuron class, mainly identified by the expression of the neuropeptide SST. There are three major SST subclasses: Martinotti, non-Martinotti and long-projecting. Martinotti cells are a transcriptionally diverse group, whose axon arborizes layer 1, while their soma lies mainly in layers 2,3 and 5 (Y. Wang et al., 2004; Xu et al., 2006). Non-Martinotti cells don't have ascending axon to layer 1, mainly populate layer 4, and they target locally basket PV interneurons (Nigro et al., 2018; Xu et al., 2013; Xu et al., 2006). Projection neurons populate layer 6 and project to other cortical areas. They exhibit irregular firing patterns and often co-express Nos, Chodl and NPY (He et al., 2016). A recent study demonstrated that SST INs separate during embryonic stages into projection neurons and Martinotti cells/non-Martinotti cells with diverging developmental trajectories (Fisher et al., 2024). Many SST subtypes can be already observed in the embryo, although diversity is further developed during early postnatal days. Recent single-cell sequencing studies utilizing both mouse and human samples have identified more subclasses for SST INs, marking SST as the most diverse IN group (Bakken et al., 2018; Hodge et al., 2019; Tasic et al., 2016; Tasic et al., 2018).

During the first postnatal week, thalamic axons target SST interneurons of layer 5 of somatosensory cortex. In turn, SST interneurons give strong input on PNs and the immature PV cells (Marques-Smith et al., 2016). Disruption of this early network results in defective maturation of the thalamocortical circuit, making SST interneurons very crucial in thalamocortical input maturation (Marques-Smith et al., 2016). SST interneurons require Cbln4 to form synapses. Other surface proteins such as Protocadherin-18 are specifically expressed in SST cells (Favuzzi et al., 2019).

PV and SST interneurons in disease

Cortical interneuron dysfunction has been involved in the pathology of neurodevelopmental disorders, such as autism spectrum disorders, epilepsy, schizophrenia and Alzheimer's disease with synaptic connectivity impairments being the main contributor in the pathology (Contractor et al., 2021; Leitch, 2024; Marin, 2012, 2024). The recent findings of the prolonged development of cortical interneurons in humans, including late neurogenesis and migration during the first postnatal months, may have important clinical implications. Third-trimester complications may influence neurogenesis and migration of cortical interneurons, especially those destined to the frontal and temporal cortices. (Marin, 2025).

Autism spectrum disorders

One of the first theories of autism spectrum disorders was the increased ratio of excitation to inhibition (E/I), due to cortical interneurons dysfunction (Hussman, 2001). PV INs seem to be the main IN subtype involved in the pathophysiology of autism, as numerous studies in mouse models and human brains suggest that hypofunction of PV INs is crucial to the pathogenesis (Contractor et al., 2021). Other IN types could also be involved in the pathophysiology of autism, but they remain to be investigated. Genetic models of autism have shown defects in GABAergic INs. The number of cortical PV interneurons is reduced in the *Cntnap2*^{-/-} mouse model, (Penagarikano et al., 2011; Vogt et al., 2018). *SHANK3* is a risk gene of autism. In *Shank3* mutant mouse models, cortical connectivity and PV protein levels are reduced (Filice et al., 2016; Pagani et al., 2019). *RELN* gene is also an autism risk gene. In the reelin heterozygous mice, the number of GAD67+ interneurons is reduced in the PFC (Liu et al., 2001). PV-Lmo4 knock-out mice display autism-like behaviours and reformed anterior cingulate cortical circuits (Zhang et al., 2020)

Epilepsy

Dysregulated inhibition is a common discovery in epilepsy patients and animal models (Treiman, 2001). The reduction of GABAergic-related and parvalbumin protein in the hippocampal granule cells has been observed in clinical studies and experimental epilepsy models (Godoy et al., 2022). Silencing of both PV and SST interneurons in the hippocampus results in the occurrence of spontaneous seizures in mice (Drexel et al., 2022; Drexel et al., 2017). Temporal lobe epilepsy (TLE) is a common epilepsy, described by the loss of GABAergic inhibitory interneurons and sprouting of mossy fibers in the hippocampus (Sterlini et al., 2020). Dravet syndrome is a severe epilepsy syndrome, where most of the patients have *de novo* heterozygous mutations in the *SCN1A* gene, encoding for the Nav1.1 subunit. Mouse models show that Nav1.1 is highly expressed in GABAergic interneurons, especially in PV and SST interneurons. Decreased excitability in these neurons, due

to the mutation, causes electrical imbalance and triggers epileptic seizures. (Sterlini et al., 2020).

Schizophrenia

Accumulating evidence suggests that impairments in several GABAergic components, including GAD67, the GABA transporter GAT1 and subunits of GABA_A receptor, may contribute to the clinical manifestations of schizophrenia (Lewis et al., 2005). Reduction in specific markers suggests a pronounced involvement of PV basket cells in the pathophysiology of the disease. The levels of GAD67 and PV are preferentially reduced in PV basket cells in schizophrenia patients. Given the activity-dependent expression of PV and GAD67 and maturation of PV interneurons, NMDA hypofunction have been proposed as one of the mechanisms accounting for these observations. In line with this, the density of excitatory neurons targeting PV interneurons in the prefrontal cortex is lower in schizophrenia patients compared to controls (Lewis et al., 2005; Marin, 2024).

It should be noted that although research regarding schizophrenia focuses on PV interneurons, SST interneurons are also widely affected in the prefrontal cortex of schizophrenia patients. A recent study has demonstrated reduced SST interneuron density in schizophrenia patients, accompanied by laminar deficits and increased expression of death receptor (Joshi et al., 2015). This means that SST interneurons are also vulnerable, potentially resulting in neuronal loss (specifically in layer 2 of the cortex). There is additional convincing evidence for the alterations of *SST* mRNA expression in schizophrenia. Multiple studies demonstrate a significant decrease in *SST* mRNA expression in the PFC and hippocampus in schizophrenia, often accompanied by decreased number or density of SST⁺ cells (Vid Prkacin et al., 2023).

Generating interneurons for cell replacement therapies

Direct cell reprogramming

It was thought that as cells differentiate, their fate is tightly regulated and becomes gradually restricted. This was illustrated by Conrad Hal Waddington, who used the infamous metaphor of a ball rolling down a slope, following just one pathway to get to the bottom of the slope (a terminally differentiated cell) (Waddington, 1957). However, a study in 1987 showed that overexpression of MYOD, a transcription factor normally expressed in skeletal muscle cells, converts mouse embryonic fibroblasts (MEFs) to myoblasts, suggesting that cell fates are more “plastic” (Davis et al., 1987). The landmark discovery of iPSCs, where somatic cells can be reprogrammed into pluripotency state (Takahashi & Yamanaka, 2016), encouraged

many scientists to attempt to convert somatic cells into other somatic cells. In the past decades many studies have shown that it is possible to switch cell fate by the forced expression of transcription factors and/or non-coding RNAs or through the delivery of small molecules. This process is called ‘direct reprogramming’ or ‘transdifferentiation’ (Wang et al., 2021).

Direct reprogramming holds many advantages. It enables *in situ* reprogramming of somatic cells, tackling challenges of iPSC technology, such as *ex vivo* reprogramming and large-scale expansion (Wang et al., 2021). Direct reprogramming skips intermediate or multipotent steps. Furthermore, it constitutes a faster way to generate reprogrammed cells, accompanied by reduced risk of tumorigenesis (Pereira et al., 2019). Epigenetic marks of the cell of origin are maintained, making direct reprogramming an appealing approach for disease modelling, especially for diseases associated with aging i.e. Alzheimer’s. *In situ* direct reprogramming has been applied to generate a variety of cell types *in vivo*, such as pancreatic beta cells, cardiomyocytes, neurons and hepatocytes. *In vivo* reprogramming provides the tissue-specific microenvironment, facilitating the generation of more mature cells (Wang et al., 2021).

Cell type-specific transcription factors are key for successful reprogramming into the desired cell type. Pioneer transcription factors are a group of transcription factors that open the chromatin, allowing other transcription factors to bind (Iwafuchi-Doi & Zaret, 2014). For example, Ascl1 is a pioneer factor in neuronal reprogramming and is considered an “on-target” factor, as it binds to lineage-specific loci, regardless of their chromatin state (Wapinski et al., 2013). Transcription factors can cooperate to activate or silence gene expression and facilitate reprogramming. Moreover, they influence the epigenetic landscape of the starting cell type. For example, they close the open chromatin regions of the starting cells and open the chromatin to the loci of interest (Wang et al., 2021).

Advancements in single-cell omics technology has assisted in profiling reprogrammed cells at high throughput and offer valuable insights into the molecular trajectory of direct reprogramming. This technology has helped us analyse heterogenous populations generated by direct reprogramming and dissect their reprogramming process. Unsupervised clustering has also revealed heterogeneity in starting cell populations and differences in cell cycle genes among these populations (Karow et al., 2018; Treutlein et al., 2016). Trajectory analysis is central for the reconstruction of cellular dynamics during reprogramming process. Such analysis demonstrated that early phases determine the reprogramming success and that cells pass through novel cell states (Karow et al., 2018; Treutlein et al., 2016).

Neuronal direct reprogramming

The discovery that radial glial cells can generate neurons in the developing murine forebrain (Noctor et al., 2001) gave the idea that other glial cell types can be converted into neurons by ectopically expressing neuronal transcription factors. Indeed, overexpression of the transcription factor PAX6 is adequate to switch fate in postnatal astrocytes *in vitro* (Heins et al., 2002). Glial cells have been used for neuronal reprogramming. Human pericytes have been converted to neurons with *Ascl1* and *Sox2* (Karow et al., 2018). Mouse microglia have been used for conversion into neurons (Matsuda et al., 2019). Additionally, human astrocytes can be effectively reprogrammed into glutamatergic neurons by *NeuroD1* (Guo et al., 2014) and *Neurogenin2* (Masserdotti et al., 2015). The easy accessibility of human fibroblasts opened a new avenue in neuronal direct reprogramming (Bocchi et al., 2022). Two landmark studies show that human and mouse fibroblasts could be converted into functional neurons, by overexpressing *Ascl1*, *Brn2*, and *Myt1l* (ABM) (Pang et al., 2011; Vierbuchen et al., 2010). The same combination has also been used for *in vivo* conversion of fibroblasts to NeuN-express cells (Torper et al., 2013). Human subtype specific neurons can be generated via directed reprogramming by using different TF combinations and starting cell populations. A notable study demonstrated that MEFs and human adult fibroblasts can be reprogrammed in dopaminergic neurons by overexpressing *Ascl1*, *Nurr1* and *Lmx1a* (ALN) (Caiazzo et al., 2011). Also, human astrocytes and glial progenitor cells are reprogrammed to dopaminergic neurons using the same combination (Addis et al., 2011; Nolbrant et al., 2020). Human fibroblasts can be reprogrammed to dopaminergic neurons by overexpressing ABM+FoxA+Lmx1a (Pfisterer et al., 2011). Overexpressing *NEUROD1*, *ASCL1*, *LMX1A*, and *mi-R218* under the tetracycline-dependent system (TetO) results in generation of dopaminergic neurons in human astrocytes and the 6-OHDA-induced Parkinson's disease (PD) mouse model (Rivetti di Val Cervo et al., 2017). Subtype-specific GABAergic neurons have been generated from mouse and adult human fibroblast by delivering *Ascl1*, *Dlx5*, *Lhx6*, *Sox2* and *Foxg1* (Colasante et al., 2015). Additionally, *Ascl1* with forskolin has converted mouse fibroblasts into interneurons (Shi et al., 2016). Human fibroblasts have been converted to cholinergic motor neurons with *NEUROG2*, *SOX11*, *ISL1*, and *LHX3* (M. L. Liu et al., 2013). Furthermore, human fibroblasts have been converted into medium spiny neurons by overexpressing *miR-124*, *miR-9/9* with *CTIP2*, *DLX1*, *DLX2*, and *MYT1L* (Victor et al., 2014).

Glia-to-interneuron reprogramming

Starting population for cell reprogramming is very important for a successful outcome. Astrocytes and NG2 glia (or oligodendrocyte precursor cells: OPCs or glial progenitor cells: GPCs) have been extensively used for neuronal reprogramming. Astrocytes are spread throughout the brain and exhibit proliferative

potential after injury (Robel et al., 2011). They appear to be transcriptionally heterogeneous across brain regions and therefore have different reprogramming capacity, depending on the region (Chouchane et al., 2017; Heinrich et al., 2010; Herrero-Navarro et al., 2021; Hu et al., 2019; Mattugini et al., 2019). NG2 glia reside in the brain parenchyma and proliferate throughout lifespan, making this population an interesting candidate for starting reprogramming, reducing the risk of depletion upon reprogramming (Hughes et al., 2013). Interestingly, NG2 glial are more prone to reprogramming toward interneurons, possibly due to their common origin (Miyoshi et al., 2007), regardless of the transcription factor combination. Notably, mouse striatal NG2 glial has been successfully reprogrammed into GABAergic interneurons of PV phenotype by delivering the ALN combination (Pereira et al., 2017; Torper et al., 2015). Astrocytes demonstrate limited capability of generating interneurons and they are more prone to glutamatergic fate, due their common developmental origin (Dimou & Gotz, 2014) Their regional identity plays a crucial role in their fate acquisition.

There are numerous studies reporting both *in situ* and *in vitro* generation of GABAergic interneurons from glial cells, utilizing various combinations of transcription factors. Transduction of cortical mouse astroglia with Dlx2 alone or in combination with Ascl1 can generate interneurons (Heinrich et al., 2010; Masserdotti et al., 2015). NG2 glia can be converted to GABAergic interneurons by overexpressing NeuroD1 (Guo et al., 2014). Delivery of Sox2 alone can generate GABAergic interneurons (Heinrich et al., 2014). Interestingly, cerebellar astrocytes reprogramme towards interneurons with Ascl1 or Ngn2 (Chouchane et al., 2017). Reactive glia in epileptic hippocampus can convert into GABAergic interneurons with NeuroD1 (Zheng et al., 2022) and with Ascl1/Dlx2 combination (Lentini et al., 2021). Subtype specific interneurons can also be generated *in vivo*. Delivery of Phospho-deficient Ascl1 and Bcl2 into the mouse brain converts glial cells into GABAergic neurons with PV interneuron characteristics (Marichal et al., 2024). All these studies utilize murine cells. Nonetheless, human systems are critical for generating interneurons of human source that have clinical potential. Human astrocytes can be converted into functional GABAergic interneurons by Ascl1 (Masserdotti et al., 2015). In our lab, we have demonstrated that human glial progenitor cells can be successfully converted into GABAergic interneurons of PV phenotype (Giacomoni et al., 2020).

Generating interneurons for disease modelling

Disease modelling using induced pluripotent stem cells (iPSCs)

Since its conception in 2006, iPSCs technology has been fundamental and widely used in a variety of applications, such as cell therapy, cancer immunotherapy and disease modelling. The human origin of iPSCs and the ability to obtain patient-specific cells are the main advantages of this technology. These models may reveal human-specific disease mechanisms, difficult to identify in animal models (Okano & Morimoto, 2022). Furthermore, the increasing complexity of iPSC-based cellular models, such as organoids and assembloids that contain multiple cell types and mimic original cytoarchitecture, have advanced our knowledge about normal development and disease mechanisms (Takebe & Wells, 2019).

Takahashi and Yamanaka showed that overexpression of four TFs (Oct4, Sox2, Klf4, and Myc, known as OSKM or Yamanaka factors) was adequate to induce pluripotency on MEFs. Notably, these mouse iPSCs exhibited characteristics comparable to ESCs, including biological potency, gene expression profile, and epigenetic signature (Takahashi & Yamanaka, 2006). A year later, Yamanaka and Thomson independently demonstrated that pluripotency could be also induced in human fibroblasts. Yamanaka used the OSKM factors (Takahashi et al., 2007), while Thomson used OCT4, SOX2, NANOG, and LIN28 (Yu et al., 2007). These combinations are extensively used until today. Despite the high similarity with ESCs, iPSCs remain distinct from ESCs, mainly due to retained features of somatic cell identity. Incomplete erasure of somatic cell-specific epigenetic markers can affect the differentiation capabilities of iPSCs (Kim et al., 2010). Addition of small molecules targeting chromatin modifiers enables erasure of the remaining chromatin markers, improving the differentiation efficiency of iPSC (Kim et al., 2010).

The most common application of iPSCs technology is in disease modelling (Soldner & Jaenisch, 2012). Their main advantage is the ability to generate them from patients affected from the disease of interest, carrying disease mutations or genetic risk factors (Cerneckis et al., 2024). Moreover, disease-causing mutations can be engineered by CRISPR/Cas9 gene editing, generating isogenic cell lines. They can also be generated by correcting disease-causing mutations in patient-derived iPSCs to obtain a wild-type control iPSC line (Hendriks et al., 2020).

Interneuron differentiation

The importance of MGE-derived interneurons in development and disease has led the researchers to develop protocols for human stem cell-derived MGE-like cortical interneurons to study development and relevant disease mechanisms and utilize them for cell therapies. There are numerous protocols for robust generation of high-

purity MGE-like progenitors from ESCs and iPSCs (Bershteyn et al., 2023; Birey et al., 2017; Kim et al., 2014; Y. Liu et al., 2013; Maroof et al., 2013; Ni et al., 2019; Nicholas et al., 2013; Xiang et al., 2017). A common feature in these protocols is the use of dual SMAD inhibition for neuronal fate induction and activation of Shh pathway for ventral forebrain fate acquisition. While these cells mostly differentiate toward SST interneurons, obtaining PV interneurons *in vitro* has been notoriously difficult and cells should be transplanted to acquire PV fate. There are a few factors that contribute to this. One is their late development and other is the lack of important developmental cues *in vitro*, such as glutamatergic input. Recently, there have been advancements in deriving human fast-spiking PV interneurons *in vitro*, by taking advantage of the complex organoid and assembloid systems (Varela et al., 2025; Walsh et al., 2025). In these protocols, the earliest emergence of PV is around 120 days in culture, which is consistent with their late normal development.

Patient iPSC-derived interneurons

In vitro modelling of neurodevelopmental and psychiatric disorders primarily reveals molecular and cellular disease hallmarks, and they cannot be easily recapitulated in animal models due to species differences and higher complexity of the human brain, delaying the development of therapeutic treatments. iPSC-based cellular models can provide important insights into disease mechanisms and complex cellular models, such as organoids and assembloids, pave the way for studying complex disease phenotypes and advance new therapeutic strategies (Cerneckis et al., 2024).

Interneurons play a major role in the pathogenesis of neurodevelopmental and psychiatric disorders, such as epilepsy, autism spectrum disorder and schizophrenia. Therefore, there have been attempts to generate patient-specific interneurons to study disease mechanisms and discover therapeutic targets.

Autism spectrum disorder

Neural progenitor cells derived from iPSCs of patients with the autism exhibit increased proliferation and impaired migration (Wang et al., 2020). Brain organoids generated from iPSCs of patients with autism exhibit abnormal proliferation of neural progenitor cells and excessive production of interneurons (Mariani et al., 2015). A study utilizing assembloids, shows that Timothy syndrome-derived GABAergic interneurons exhibit increased saltation frequency and decreased saltation length. This phenotype was rescued by L-type calcium channel blockers (Chen et al., 2024). Another study investigating CHD8-related deficiencies shows upregulation of DLX genes (Wang et al., 2017).

Epilepsy

Dravet syndrome is the most investigated iPSC-based model of epilepsy. Studies generating patient-derived inhibitory neurons demonstrate neuronal hypoexcitability and transcriptomic changes (Sterlini et al., 2020). In one study, researchers generated a human cerebral organoid model for tuberous sclerosis complex (TSC) and identified a specific neural stem cell type called caudal late interneuron progenitor (CLIP) cells. In TSC, CLIP cells over-proliferate, generating excessive interneurons, brain tumours, and cortical abnormalities (Eichmuller et al., 2022). TLE is difficult to recapitulate in iPSC models, because there is no information on the aetiology and excessive circuit remodelling is occurring. Therefore, researchers have focused on grafting iPSC-derived cells in TLE animal models to study the pathophysiology of the disease (Sterlini et al., 2020).

Schizophrenia

Neurons derived from iPSCs of schizophrenic patients exhibit impaired neuronal complexity and excitability (Dubonyte et al., 2022). Interneuron-based cellular models of schizophrenia show dysfunctional arborization of cortical interneurons (Shao et al., 2019). Other studies show functional defects of schizophrenic iPSCs-derived INs (Kathuria et al., 2019) (Roussos et al., 2016). Notably, one study shows that schizophrenic interneurons have perturbations in genes regarding apoptosis, cell cycle, survival and MAPK signalling pathways in a 22q11.2 deletion model (Lin et al., 2016). A few studies show that schizophrenia interneurons show dysregulated mitochondrial function both alone and in microglia co-culture (Ni et al., 2020; Park et al., 2020). A recent study using dorso-ventral cortical assembloids shows migratory defects in INs with 22q11.2 deletion and defects in PV basket cells in 15q13 deletion, accompanied by impaired network oscillations (Walsh et al., 2025).

Use of generative AI in thesis

This thesis has been partially written with the assistance of the generative AI model ChatGPT. I have carefully reviewed all generated content and take full responsibility for the final content. Details of the usage of the AI model used can be found in the Key Experimental Methods section.

Aims of the thesis

The thesis includes the following specific aims:

- To generate human PV interneurons *in vitro* through glia-to-neuron reprogramming and define their lineage trajectory (Paper I).
- To evaluate the survival, integration, and functional maturation of human reprogrammed interneurons in the mouse brain, establishing their potential for future therapeutic applications (Paper II).
- To develop a 3D co-culture platform enabling hESC differentiation toward SST interneurons and to characterize their long-term maturation and subclass-specific identities (Paper III).
- To derive patient-specific SST interneurons from schizophrenia iPSCs and investigate disease-associated alterations in protein expression and neuronal function (Paper IV).

Key experimental methods

Cell culture

ESC and iPSC culture

The ESC lines used were RC17 (RCe021-A) for Papers I, II and III and H9 (WAe009-A) for Paper III. Both cell lines were cultured on Laminin 521-coated plates ($0.5 \mu\text{g}/\text{cm}^2$; Biolamina) and kept in iPS Brew XF medium (Miltenyi Biotec). Cells were split when they reached 80% confluency using EDTA (0.5 mM) and seeded in medium + $10 \mu\text{M}$ Y27632 (ROCK inhibitor; Miltenyi). Cells were passaged 3 times post-thawing before the initiation of experiments.

The iPSC lines used in Paper IV were generated in (Hathy et al., 2020; Hathy et al., 2021). Cells were cultured on Matrigel-coated plates (#354277, Corning) and kept in mTeSR medium (STEMCELL Technologies). Cells were split when they reached 80% confluency using Accutase (StemPro, Thermo Fisher) and seeded in medium + $10 \mu\text{M}$ Y27632 (ROCK inhibitor; Miltenyi). Cells were passaged 3 times post-thawing before the initiation of experiments.

Glial differentiation

Human glial progenitor cells (hGPCs) used in Papers I, II and III were generated from RC17 (RCe021-A) according to (Nolbrant et al., 2020; Wang et al., 2013). Briefly, the ESCs were detached from the plate using EDTA (0.5 mM), dissociated and kept in iPS-Brew XF medium, supplemented with Rock inhibitor in ultra-low attachment (ULA) flasks (#CLS3815, Corning) for embryoid body (EB) formation, marking day 0 of differentiation. Neuronal induction started on day 5, by switching the media to Neuronal Induction media, consisting of DMEM/F12 basal medium (Thermo Fisher), MEM Non-Essential Amino Acids (NEAA) Solution (Thermo Fisher), N2 supplement (Thermo Fisher), and Antibiotic-Antimycotic (Ab/Am, Thermo Fisher), and supplemented with bFGF ($20 \text{ ng}/\text{ml}$, Sigma-Aldrich) and Heparin ($2 \mu\text{g}/\text{ml}$, STEMCELL Technologies). EBs were attached on PLO/Lam-coated 6-well plates (#CLS3516, Corning) on day 9, to allow neural rosette formation, and on day 11 Neuronal Induction media was supplemented with retinoic acid ($0.1 \mu\text{M}$, Sigma-Aldrich). On day 16 the medium was switched to Neuronal

Induction media/B27 medium (DMEM/F12, MEM NEAA, N2, B27[Thermo Fisher], Ab/Am), supplemented with retinoic acid + Purmorphamine (1 μ M, Millipore). On day 28, the cells were detached and seeded in ULA 6-well plates (#CLS3471) for floating clusters' formation. After the detachment, clusters were kept in Neuronal Induction media/B27 medium, supplemented with Purmorphamine and bFGF (10 ng/ml). On day 37, the medium was switched to glial medium (GM), consisting of DMEM/F12 basal medium, B27 supplement, N1 supplement (Sigma-Aldrich), MEM NEAA, Ab/Am, T3 (60 ng/ml, Sigma-Aldrich), db-cAMP (1 μ M, Sigma-Aldrich), Biotin (100 ng/ml), PDGF-AA (10 ng/ml, R&D Systems), IGF-I (10 ng/ml, R&D Systems), NT-3 (10 ng/ml, R&D Systems) and Purmorphamine. On day 55 Purmorphamine was removed from the medium. Around day 70, clusters were manually cut using disposable sterile scalpels under a dissection microscope and seeded onto PLO/Lam coated 6-well plates (#CLS3516, Corning). This procedure was repeated every 30 days. At every passage, the cells were assessed by FACS for CD44 and CD140a.

Neuronal 3D reprogramming

For Paper I, hGPCs were detached with a cell scraper and dissociated into single cells with Accutase (StemPro, Thermo Fisher). 100,000 single hGPCs were mixed with the lentiviral reprogramming cocktail [multiplicity of infection (MOI) of 1–2 per vector] in a total of 60 μ l of GM and seeded in 96-well ULA plates (#CLS7007, Corning) to self-aggregate. After 24 hours, 60 μ l of fresh GM containing doxycycline (5 μ g/ml, Duchefa) were exchanged to induce the expression of the tetOn-regulated transgenes. Two days later, media was changed to neural differentiation (ND) medium, consisting of NDiff227 (Takara-Clontech), supplemented with doxycycline, small molecules:CHIR99021(2 mM, Axon), SB-431542(10 mM, Axon), Noggin (0.5 mg/ml, R&D Systems), LDN-193189 (0.5 mM, Axon), VPA(1 mM, Merck Millipore) and growth factors: LM-22A4 (2 mM, R&D Systems), GDNF (2 ng/ml, R&D Systems), NT3 (10 ng/ml, R&D Systems), db-cAMP (0.5 mM, Sigma-Aldrich) Small molecules and doxycycline were removed from the medium two weeks post-transduction, to promote maturation of the neurons.

For Paper II, a similar approach was followed. In this case, 1.2 million hGPCs were mixed with the lentiviral vectors in at volume of 1ml and seeded into each well of an AggreWell™ 800 plate (STEMCELL Technologies), resulting in spheroids containing approximately 4,000 cells each.

Interneuron differentiation and co-culture

Interneurons in Papers III and IV were generated from RC17, H9 and iPSC lines according to (Nicholas et al., 2013). Briefly, on day 0, cells were detached using EDTA or Accutase (See ESC and iPSC culture section) and seeded in 96-ULA plates (#CLS7007, Corning) in a density of 10,000 cells/well in medium + Rock inhibitor for EB formation. On day 1, medium was switched to basic interneuron medium containing Neurobasal-A (Thermo Fisher), supplemented with B27 +Vitamin A (Thermo Fisher), MEM NEAA, GlutaMAX (Thermo Fisher), Ab/Am and the following patterning factors: SB-431542, Noggin, DKK1 (100 ng/ml, R&D Systems) and Purmorphamine. On day 9, EBs were attached on PLO/Lam coated plates (#CLS3516, Corning) and on day 15 SB, Noggin, DKK1 were removed from the media. On day 25, cells were detached and dissociated, and single cells were plated on PLO/lam coated wells (#CLS3516, Corning) for terminal differentiation. Two days later, Compound E (1 mM, STEMCELL Technologies) was supplemented to the medium to push the cells out of the cell cycle for 3 media changes.

For Paper III, interneurons on day 35 were detached using Accutase, and 90,000 or 100,000 cells/well were seeded in ULA plates (#CLS7007, Corning), together with either 10,000 mouse astrocytes (mAST, ScienCell) or 10,000 hGPCs (See Glia Differentiation section) to initiate 3D co-culturing. Spheroids were then kept in basic interneuron medium supplemented with BDNF (25 ng/ml, R&D Systems R&D Systems).

For Paper IV, stem cell-derived human astrocytes were seeded on PLO/Lam-coated coverslips. Two days later, they were treated with 2 mM Arac (Sigma-Aldrich) for 24 hours, to kill proliferating cells before the addition on neurons. On day 35 of interneuron differentiation, cells were plated on top of the Arac-treated astrocytes in 2:1 ratio and kept in basic interneuron medium, supplemented with Purmorphamine, BDNF and 0.1% FBS (Thermo Fisher) to support astrocyte viability.

Analysis methods

RT-qPCR

Cells were lysed with RLT buffer (Qiagen), and total RNA extraction was performed using RNeasy Micro Kit (Qiagen). This was followed by reverse transcription to cDNA with Maxima First Strand cDNA Synthesis Kit (Thermo Fisher). For RT-qPCR, everything was pre-mixed on a Bravo Automated Liquid Handling Platform (Agilent). cDNA (1 µl) was mixed with LightCycler 480 SYBR

Green I Master (5 μ l, Roche) and relevant primers (4 μ l, Eurofins Genomics, See Table 1) in each well of 384-well plate in 3 technical replicates for each sample. RT-qPCR was performed in a LightCycler 480 II instrument (Roche) with a 40 cycles two-step PCR protocol (95°C, 30s denaturation and 60°C, 1 min annealing/elongation). Analysis was performed from technical triplicates and relative gene expression was normalized against the housekeeping genes *GAPDH* and *ACTB* and calculated using the comparative CT Method ($\Delta\Delta$ CT Method). Every sample was compared to cells pre-conversion or pre-differentiation.

Tabel 1. List of primers used in the thesis
Primers with their sequence.

Gene	Gene name	Primers (forward-reverse)
<i>ACTB</i>	Beta-actin	CCTTGCACATGCCGGAG CCTTGCACATGCCGGAG
<i>ASCL1</i>	Achaete-Scute Family BHLH transcription factor 1	CTAAAGATGCAGGTTGTGCG GGAGCTTCTCGACTTCACCA
<i>Ascl1-WPRE</i>	Achaete-Scute Family BHLH Transcription Factor 1	CTAAAGATGCAGGTTGTGCG GGAGCTTCTCGACTTCACCA
<i>ARX</i>	Aristeless Related Homeobox	CTGAGCACATTTCTCTGGAGCG TGGAAAGAGCCTGCCGAATGCC
<i>CALB1</i>	Calbindin 1	TGGTCCATTTTCGACGCTGACG ATCCAGCCTTCTTTTCGCGCTG
<i>CALB2</i>	Calbindin 2	TGGAGGCTTGGCGGAAGTACGA CCGGTTCCGCTTCTTCAGCAGG
<i>CBLN4</i>	Cerebellin 4 precursor	CCGAGATGAGCAACAAGACG CCCCCGCAAGGCAGATATT
<i>CCK</i>	Cholecystokinin	AGGTTATCGACAGAAACGGA CTTATCCTGTGGCTGGGGTC
<i>CDK6</i>	Cyclin dependent kinase 6	TGCACAGTGTACAGAACAGA ACTATAGATCGGGCAAGGC
<i>CHODL</i>	Chondrolectin	ACAGATGAACCTTCTCTGCGG TCTGATGGGTCTCTCTGGT
<i>DLX2</i>	Distal-Less Homeobox 2	ACAGACCTCGGATCCGCC CTGCGGGGTCTGAGTGGGGT
<i>DLX5</i>	Distal-Less Homeobox 5	GCCTCCGGGACACTCTATT CAGTTTCCGAACCTTCCCCAT
<i>DLX5-WPRE</i>	Distal-Less Homeobox 5-viral	GGCTCCGGGACACTCTATT AGCAGCGTATCCACATAGCG
<i>FOXG1</i>	Forkhead Box G1	CTGTCTGGGGACTGCTGAT GTCTGGTCCCAGGGATGTT
<i>FOXG1-WPRE</i>	Forkhead Box G1-viral	AGGGGTCTTCTCCAAACCCT GCAGCGTATCCACATAGCGT
<i>GAD2</i>	Glutamate decarboxylase 2	ATCCTCACGACTCAGCTCCC GAGCTTTAAAGAGACCCGGACT
<i>GAPDH</i>	Glyceraldehyde-3-Phosphate Dehydrogenase	TTGAGGTCAATGAAGGGGTC GAAGGTGAAGGTCCGAGTCA
<i>GFAP</i>	Glial Fibrillary Acidic Protein	TCATCGCTCAGGAGGTCCTT CTGTGCCAGAGA TGGAGGTT
<i>LXH6</i>	Lim Homeobox 6	CACCTCCGCATCTGCCGT CGCAGCTTGGACACTGGATCT
<i>LHX6-WPRE</i>	LIM Homeobox 6-viral	CGTCCACCTCAAAGCCGATA GCAGCGTATCCACATAGCGT
<i>MAF</i>	MAF BZIP Transcription Factor	CACCTGCAAGTTGGAGCCAT ACTGGTAAGTACACGATGCTGG
<i>MAFB</i>	MAF BZIP Transcription Factor B	CGCTGGCCATGGAGTATGTC GTGCTTCTGTTCCGTCGGG
<i>MAP2</i>	Microtubule Associated Protein 2	CCGTGTGGACCATGGGGCTG GTCGTGGGGGTGATGCCACG
<i>MEF2C</i>	Myocyte Enhancer Factor 2C	TCTCTCCCTGCTTCTACTCA GGAGTGGAAATTCGTTCCGGT
<i>MYH8</i>	Myosin Heavy Chain 8	AGAAGCCACTGGGCATCTTC TACGAAAGTGAGGGTGTGTGC
<i>NKX2-1</i>	NK2 Homeobox 1	AGGGCGGGGCACAGATTGGA GCTGGCAGAGTGTGCCCAGA
<i>NPY</i>	Neuropeptide Y	TGTTCCAGAACTCGGCTTG TGCATTGGTAGGATGGGTGG

Gene	Gene name	Primers (forward-reverse)
<i>PDGFRA</i>	Platelet-Derived Growth Factor alpha	CCTTGGTGGCACCCCTTAC TCCGTACCCACTCTTGATCTT
<i>POU2F3</i>	POU Class 2 Homeobox 3	ATGCGGGCTCCTTTAACCGG TTAGACGCTGCGGTGCGCATG
<i>PVALB</i>	Parvalbumin	TGCAGGATGTCGATGACAGA TTTCTTCAGGCCGACCATT
<i>SLC32A1</i>	Solute Carrier Family 31 Member 1	AGATGATGAGAAACACCCCCAG CACGACAAGCCCCAAAATCAG
<i>Sox2-WPRE</i>	SRY-Box Transcription Factor 2-viral	TCGCACATGTAGGATCCAA AGCGTAAAGGAGCAACATAGT
<i>SST</i>	Somatostatin	CAAGCCGCTTTAGGAGCGAG AGGCGGCAGGACAGCATCT
<i>SYN1</i>	Synapsin 1	CCCGTGGTTGTGAAGATGGGGC TGCCACGACACTTGGGATGTCC
<i>SYN1</i>	Synaptophysin	ACCTCGGGACTCAACACCTCGG GAACCCAGGTTGCCGACCCAG
<i>TACSTD2</i>	Tumor Associated Calcium Signal Transducer 2	CCACCAACAAGATGACCGTG CAGCAGACACTTGGAGGTCA
<i>TH</i>	Tyrosine Hydroxylase	CGGGCTTCTCGGACCAGGTGTA CTCCTCGGGGTGTACTCCACA
<i>VIP</i>	Vasoactive Intestinal Peptide	TCTCACAGACTTCGGCATGG TCATTGCTCCCTCAAAGGGT

Immunoassays

Whole spheroid immunostainings and optical clearing were performed according to (Giacomoni et al., 2023). Briefly, spheroids were fixed using 4% paraformaldehyde (PFA) or a mix of 4% PFA and 0.25% glutaraldehyde (Sigma-Aldrich) for 20 min at room temperature (RT). Spheroids were incubated overnight at RT in blocking solution containing 5% donkey serum, 0.3% Triton X-100 (Sigma-Aldrich) diluted in PBS. Incubation with primary antibodies (See Table 2) diluted in blocking solution was performed for 24h-48h at RT on a shaker. Afterwards, spheroids were incubated in secondary antibodies conjugated to Alexa-488, Alexa-568 (Thermo Fisher) /Cy3, or Alexa-647 (1:200; Jackson ImmunoResearch Laboratories) and DAPI (1:2000, Sigma-Aldrich) for 24h-48h at RT on a shaker. Samples were then dehydrated in an ascending serial methanol concentration for 10 min each, and lipids were removed by incubating with a mix of dichloromethane-methanol for 1h in RT, followed by 2 washes of 10 min with dichloromethane. Finally, spheroids were flushed with ethyl cinnamate and transferred to 96 well-plates with flat and clear bottoms (Ibidi) for confocal microscopy. Spheroids generated for Paper II were not cleared.

For 2D immunostainings, cells were fixed in 4% PFA or 4% PFA+0,25% glutaraldehyde for GABA staining for 15 minutes in RT. After fixation, cells were washed 3x with PBS and stored in 4°C. Before blocking, cells were washed 3x times with PBS and incubated with blocking solution (PBS with 0.1% Triton X-100 and 5% donkey serum) for 1-3 hours in RT. After this incubation, cells were incubated with primary antibodies at 4 °C overnight (See Table 2). The next day, cells were washed 2x with PBS and incubated with fluorescent-conjugated secondary antibodies and DAPI for 1h at RT. Cells were washed 3x with PBS and kept at 4°C in PBS until imaging.

Tabel 2. List of primary antibodies used in the thesis

Primary antibodies with all the relevant information.

Antibody	Concentration	Company	Ref #
Chicken anti-DCX	1:500	Abcam	ab153668
Chicken anti-GFAP	1:2000	Merck Millipore	AB5541
Chicken anti-GFP	1:1000	Abcam	ab13970
Chicken anti-MAP2	1:2000	Abcam	ab5392
Goat anti-PDGFR α	1:300	R&D Systems	AF-307-NA
Goat anti-SOX10	1:25	R&D systems	AF2864
Mouse anti-human nuclear antigen	1:200	Millipore	MAB128
Mouse anti TUJ-1/BIII	1:1000	Biologend	801211
Mouse anti-hNCAM	1:1000	Santa Cruz	sc106
Mouse anti-MAP2	1:300	Merck Millipore	MAB 3418
Mouse anti-Nestin	1:500	BD	611658
Mouse anti-SOX2	1:50	Santa Cruz	sc-365823
Mouse anti-SST	1:200	Santa Cruz Biotechnology	sc55565
Mouse anti-TAU	1:500	Thermo Fisher. Scientific	MN1000
Rabbit anti-CALB-2	1:200	Abcam	ab702
Rabbit anti-GABA	1:2000	Sigma-Aldrich	A2052
Rabbit anti-Ki67	1:500	Abcam	ab238020
Rabbit anti-NeuN	1:500	Millipore	ABN78
Rabbit anti-PV	1:500	Swant	PV-28
Rabbit anti-TH	1:1000	Merck Millipore	AB152
Rabbit anti-TTF-1/Nkx2.1	1:500	Novus Biologicals	8G7G3

Nuclei isolation and sorting for single-nuclei RNA sequencing (snRNA-seq)

For Papers I and III, spheroids were collected, snap-frozen on dry ice and kept at -80°C until processed. Nuclei isolation was performed according to the following protocol (Sodersten et al., 2018) with modifications. Briefly, spheroids were thawed at 4°C and dissociated in ice-cold lysis buffer (0.32 M sucrose, 5 mM CaCl₂, 3 mM MgAc, 0.1 mM Na₂EDTA, 10 mM Tris-HCl pH 8.0, 1 mM DTT, 0.1% Triton X), supplemented with EDTA-free proteinase inhibitor (Roche) and RNase inhibitors (Ambion™ and SUPERaseIn™). Lysates were centrifuged for 30 min at 11.000 x g. Supernatant was discarded and the pelleted nuclei were resuspended in the

resuspension buffer (0.1% BSA Fraction V, PBS -/-, RNase inhibitors) supplemented with Draq7™ (BD Biosciences). The nuclei were filtered through a 70 µm cell strainer into BSA-coated DNA LoBind tubes (Eppendorf) for sorting. Nuclei sorting was performed with a FACS Aria cell sorter on a 100 µm nozzle and with FACSDiva software (BD Biosciences) on a low flow rate to separate single nuclei. 10.000-12.000 nuclei were collected for each sample in a total volume of 20 µl and directly processed for cDNA libraries' generation.

High-content screening

High-content screening for Papers I, III and IV was performed on Operetta High Content Imaging System using the Harmony 5.2 or software (Waltham). The measurement of the mean fluorescence for antigen-positive cells was defined above the background fluorescence of antigen-negative cells (internal negative control). For Papers I and IV, the “Neurite Outgrowth” script on Harmony 5.2 (Perkin Elmer) was used.

Imaging

Fluorescent images were taken with a Zeiss 780 Confocal Laser-Scanning Inverted Microscope (20X objective and 40X object) and with Zeiss Zen Blue Edition software or with Leica Stellaris 5 using the LAS X software (Leica). For the 40X pictures of Paper I, the HDR mode of Zeiss confocal was used. Images were edited on ImageJ (NIH) and adjustments were applied equally across the entire image without loss of information.

Proteomics data analysis

Proteomics data were further analysed and visualized in R (version 4.3.3). Data were imputed using the imputeLCMD package (version 2.1) and prcomp command was used for Principal Component Analysis. For differential protein expression, proteins with fold change > |1.5| and adjusted p value < 0.05 were considered and pheatmap package (version 1.0.13) was used for visualization. For Overrepresentation and Gene Set Enrichment Analysis (GSEA) for Gene Ontology categories (Biological Process, Molecular Function, and Cellular Component), clusterProfiler package (version 4.10.1) was used. For violin plots, ggplot (version 2.0.09) was used.

Use of generative AI models

ChatGPT was used during the writing of this thesis to support text organization and language processing. This model was used as language assistant, helping in the

synthesis of complex information, and improving the clarity and readability of the text. All content generated with ChatGPT was carefully reviewed by the author. The tool was strictly employed in a supportive role and did not influence data interpretation.

Summary of main results

A bona fide Parvalbumin Chandelier trajectory in Human Interneuron Reprogramming (Paper I)

Neuronal reprogramming of human glia in 3D cultures

Given the critical role of PV interneurons in disorders such as schizophrenia and epilepsy (Leitch, 2024; Marin, 2025), improved strategies for their generation and replacement in the diseased brain are needed. Here, we applied glia reprogramming as a novel approach to generating PV interneurons with potential for cell replacement therapies. hGPCs, generated according to (Nolbrant et al., 2020; Wang et al., 2013) (Figure 2B), were transduced with cocktail of five reprogramming factors (*Ascl1*, *DLX5*, *LHX6*, *Sox2* and *FOXG1*; ADLSF) to drive reprogramming toward interneurons in a 3D culture system (Giacomoni et al., 2024) (Figure 2A, C). Initial protein expression profiling for neuronal and glial markers showed a clear upregulation of the neuronal marker TAU (tubulin-associated protein) in the ADLSF group and downregulation of glial markers PDGFR α and GFAP (Figure 2D). The control groups remained positive for PDGFR α and GFAP (Figure 2D).

These data demonstrated a successful reprogramming of hGPCs into neurons in a 3D environment, mediated by the transduction with the ADLSF factors.

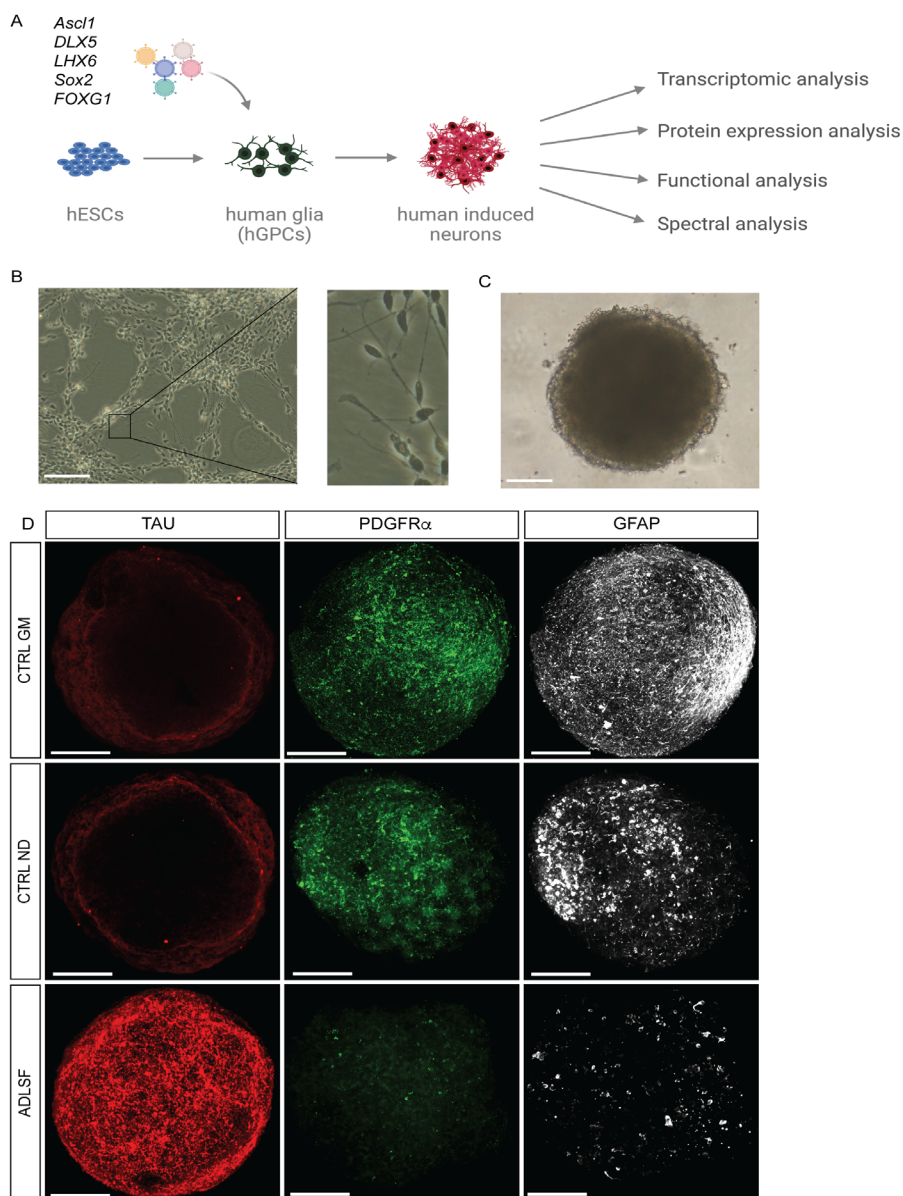


Figure 2. Reprogramming of hGPCs into neurons in a 3D environment.

(A) Schematic overview of experimental design. (B) Brightfield photo of hGPCs in 2D culture before conversion. Scale bar: 100 μ m. (C) Brightfield photo of a reprogrammed spheroid. Scale bar: 100 μ m. (D) Maximum intensity projection images of control (GM and ND) spheroids and ADLSF-transduced spheroids with TAU, PDGFR α and GFAP at day 21 or reprogramming. Scale bar: 100 μ m.

Early dynamics of glia-to-neuron reprogramming and gradual functional maturation of GABAergic interneurons

Next, we sought to investigate the transcriptional dynamics governing glia-to-neuron reprogramming. Thus, we performed 10x Genomics droplet-based single-nuclei RNA sequencing (snRNA-seq) on day 0 and days 1,3,7,13 and 21 days post-transduction. Transcriptional changes were already observed from day 3, followed by the appearance of transcriptionally new clusters at days 7-13 (Figure 3A). At day 21, a new cluster occurred which was transcriptionally closer to earlier time points (likely due to the loss of mature neurons during nuclei extraction, Figure 3A).

We further verified a glia-to-neuron transition as demonstrated by assessing the expression dynamics of glial and mature neuronal genes. Glial genes *GFAP* and *PDGFRA* were expressed at earlier time points and gradually downregulated during the reprogramming course, while the mature neuronal genes *SYN2* and *SYN3* were mainly seen from day 7 onwards (Figure 3B). This analysis showed the rapid conversion of glial cells to neurons, where transcriptionally mature neurons were already present at day 7. To prove the presence of functional neurons, we performed whole-cell patch clamp electrophysiology experiments at different time points during reprogramming. Data showed that reprogrammed neurons undergo gradual functional maturation, as their ability to fire repetitive action potentials (APs) upon current injection was improved over time with day 50 representing the most mature time point (Figure 3C).

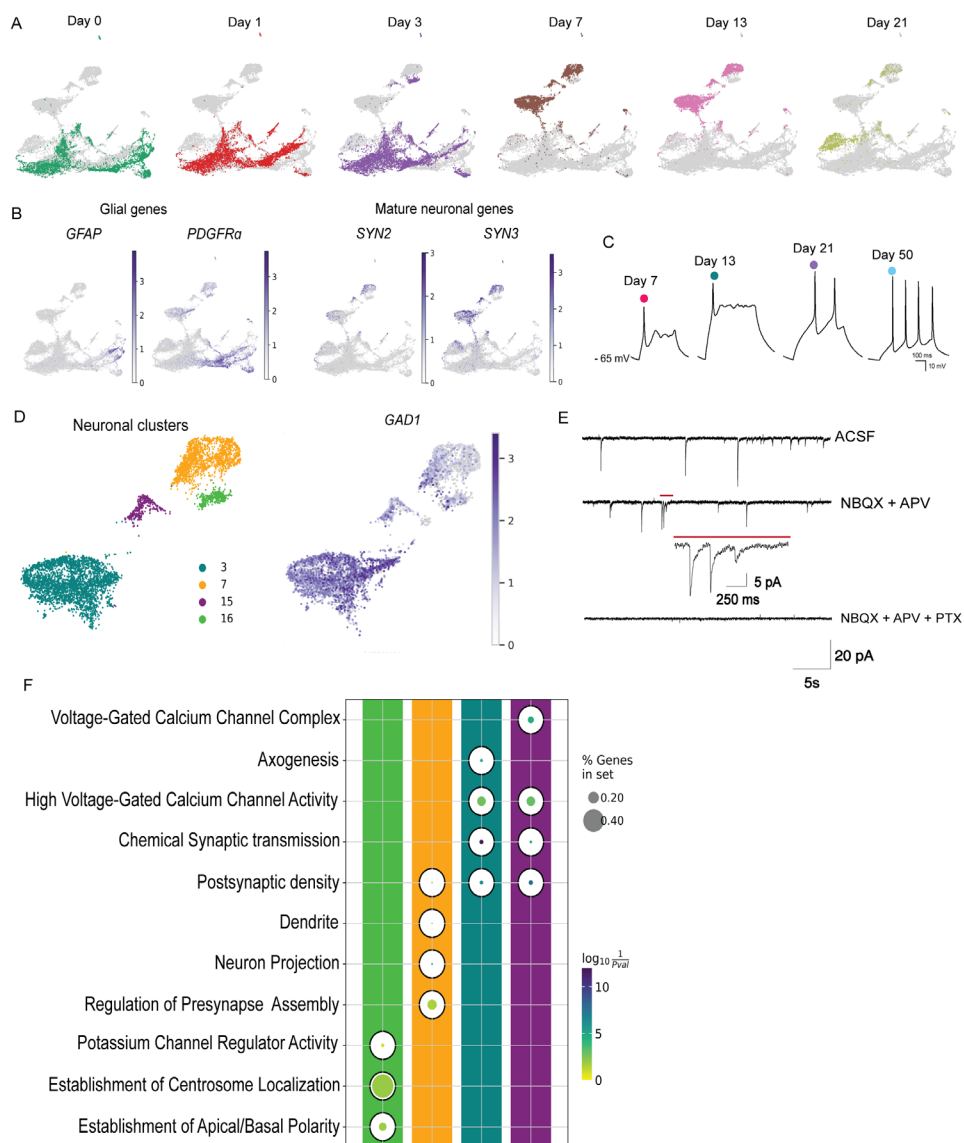


Figure 3. Early neuronal reprogramming dynamics and maturation of reprogrammed interneurons.

(A) UMAPs visualizing cells at different time points of reprogramming. (B) UMAPs visualizing the expression of glial genes (*PDGFRA* and *GFAP*) and mature neuronal genes (*SYN2* and *SYN3*) across all time points. (C) Representative traces of evoked action potential at different time points. (D) UMAP visualizing the neuronal clusters with different colors and *GAD1* expression. (E) Sample traces of postsynaptic activity blocked with 5 μ M NBQX+ 50 μ M APV and 5 μ M NBQX + 50 μ M APV+ 100 μ M PTX on day 21. (F) Pathway analysis of neuronal clusters.

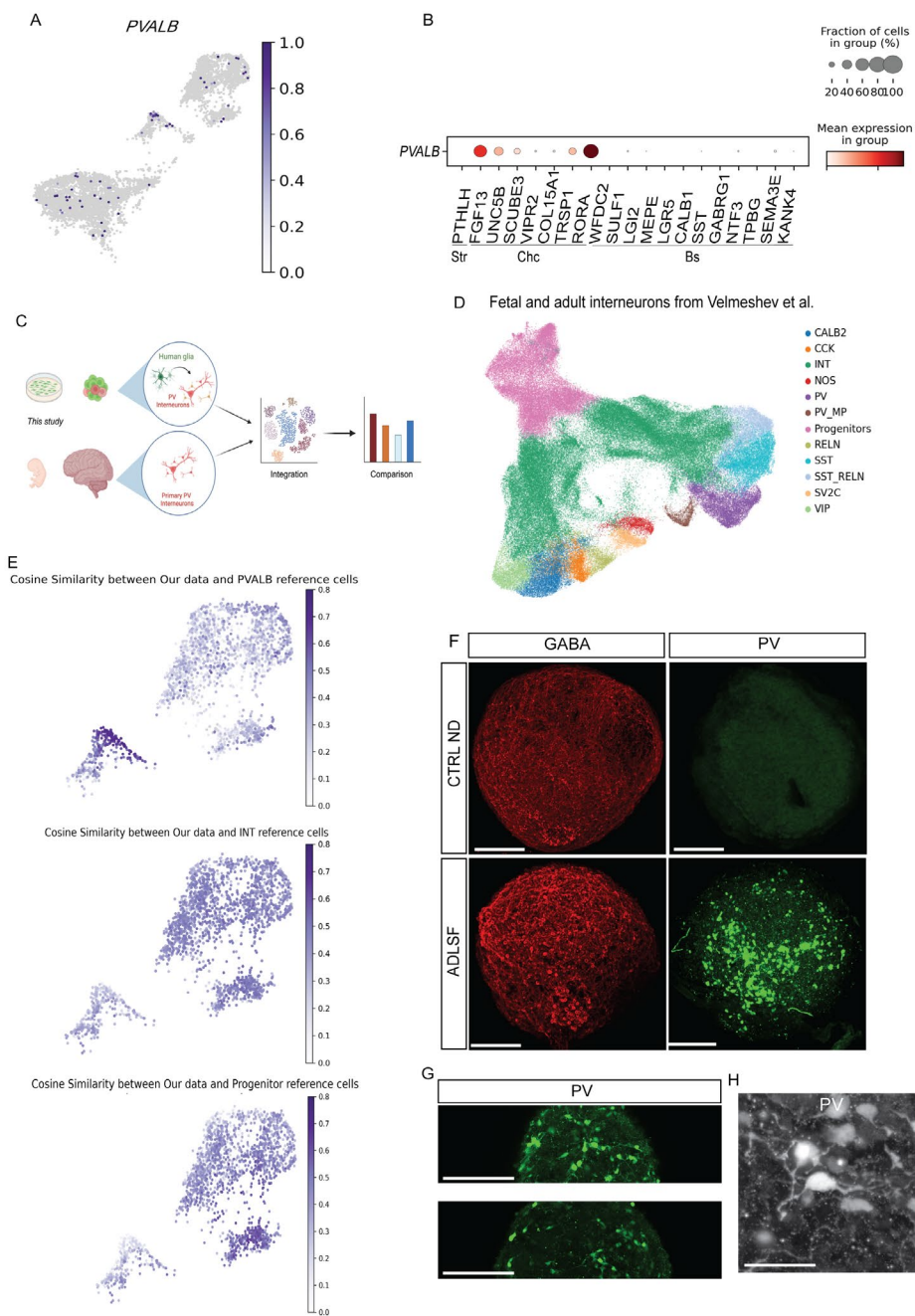
Next, we focused our analysis on the neuronal clusters present on days 3-13: 3 (dark green), 15 (purple), 7 (yellow) and 16 (lime) (Figure 3D). These clusters were all expressing the pan-GABAergic marker *GAD1* (Figure 3D), marking the presence of inhibitory GABAergic interneurons in these clusters. To further confirm that, we performed classical pharmacology experiments at day 21. Addition of glutamatergic antagonists (NBQX and D-APV) did not affect postsynaptic spontaneous activity, but it was impacted by the addition of GABA_A receptor antagonist picrotoxin (PTX), supporting the presence of functional inhibitory GABAergic interneurons in the spheroids (Figure 3E).

The four neuronal clusters identified in our dataset were further examined with Gene Set Enrichment Analysis (GSEA) pathway analysis (Figure 3F). Interestingly, each cluster was characterized by distinct sets of pathways. This analysis indicated that the four different GABAergic clusters are in different developmental stages, with cluster 15 representing the most transcriptionally mature cluster.

Collectively, these data showed the fast dynamics of glial reprogramming to transcriptionally mature GABAergic neurons, which were functionally active and establish synaptic communication.

Transcriptional equivalence of reprogrammed interneurons to *bona fide* cortical chandelier interneurons

To further explore subtypes present in the reprogrammed GABAergic neurons, we examined the expression of subtype-specific genes within the neuronal clusters. *PVALB* was the most prominent subtype-specific marker and specifically enriched in cluster 15 (Figure 4A). Furthermore, the *PVALB*⁺ cells were assessed for their subclass specification among striatal, cortical chandelier and cortical basket interneurons, each characterized by specific marker expression. Most *PVALB*-expressing cells co-expressed chandelier subclass markers, while a few co-expressed the basket subclass genes (Figure 4B). These results suggested that reprogrammed PV interneurons were mainly of chandelier subclass.



To assess whether the reprogrammed *PVALB*-expressing interneurons were equivalent to *bona fide* human cortical primary interneurons, we integrated and compared our neuronal clusters with a published dataset of cortical foetal and adult human interneurons (Velmeshev et al., 2023) (Figure 4C, D). PV clusters (PV and PV_MP) from the published dataset were more similar to cluster 15. Furthermore, “Interneurons” (INT) group was the most similar group for the yellow cluster and “Progenitors” for the lime cluster (Figure 4E). This was the first evidence that human-reprogrammed PV interneurons were transcriptionally similar to primary cortical PV interneurons.

Immunocytochemistry confirmed the presence of GABAergic interneurons on protein level. While GABA protein expression was low in ND control group, it was considerably increased in the ADLSF-transduced group at day 21 (Figure 4F). Importantly, we could observe strong induction of PV protein only in the ADLSF condition (Figure 4F), validating a PV phenotype. PV+ cells were distributed throughout the whole spheroid (Figure 4G) and exhibited elaborate neuronal morphology (Figure 4H).

These data indicated that human reprogrammed PV interneurons were *bona fide* with endogenous PV interneurons, with the remaining neuronal clusters being similar to endogenous interneuron and progenitor populations, thus representing different developmental stages.

Revealing the lineage trajectory for glia-to-chandelier reprogramming

To further dissect the dynamics of glia-to-PV conversion, we assessed the cellular trajectory using RNA velocity (Bergen et al., 2020), accompanied by PAGA (Bergen et al., 2020). Latent time was used to track maturity from a "branching point" (red cells in Figure 5A) to the PV cluster (dark blue cells in Figure. 5A). This analysis resulted in the emergence of two distinct pathways. The trajectory marked by the black arrows did not reach the PV cluster, indicated as “non-PV trajectory”, while the trajectory marked by the blue arrows, successfully reached the PV cluster and was indicated as the “PV trajectory”.

Figure 4. Equivalence of reprogrammed interneurons with endogenous cortical chandelier PV interneurons.

(A) UMAP visualizing *PVALB* expression in neuronal clusters. (B) Dot plot visualizing expression of subclass-specific markers in *PVALB*-expressing cells. (C) Schematic overview showing the integration with a published dataset of human cortical interneurons. (D) UMAP visualizing fetal and adult human cortical interneurons from a published dataset (Velmeshev et al., 2023). (E) Cosine similarity score between our neuronal clusters and reference clusters. (F) Maximum intensity projections of immunostainings of control (ND) and ADLSF-transduced cells with GABA and PV. Scale bar: 100 μ m. (G) Z-stacks from whole spheroid immunostaining, showing distribution of PV+ cells. Scale bar: 100 μ m. (H) 40X image of a PV interneuron. Scale bar: 40 μ m.

To further support these findings, we performed diffusion pseudo-time analysis, which is based on gene expression similarity (Figure 5B). We could detect the clusters involved in the PV trajectory on the diffusion map, which appeared interconnected (Figure 5C). The end of the purple cluster, corresponding to the PV cluster, showed the highest level of maturity (Figure 5D, E).

We next investigated important genes involved in the “PV trajectory” (Figure 5F). There was a gradual downregulation of hGPC-related genes, such as *MAML2*, *PTPRZ1*, *SOX6* and *GLIS3*. In contrast, there was an increased expression of mature neuronal markers, such as *NTM*, *OPCML*, *RBFOX3* and *SNAP25*, confirming the successful transition toward a mature neuronal fate. Notably, there was transient upregulation of progenitor genes, such as *NKX2-1* and neuronal migration genes *PLEKHG1* and *WWOX*, alongside with upregulation of postsynaptic genes *GPHN* and *NLGNI*. Towards the end of trajectory, we detected upregulation of genes orchestrating PV transcriptional profile (*PPARGC1A*, *ESRRG*, and *RORA*).

Altogether, we proved that human glial cells followed a specific trajectory for PV fate acquisition, where key genes contributed to this process.

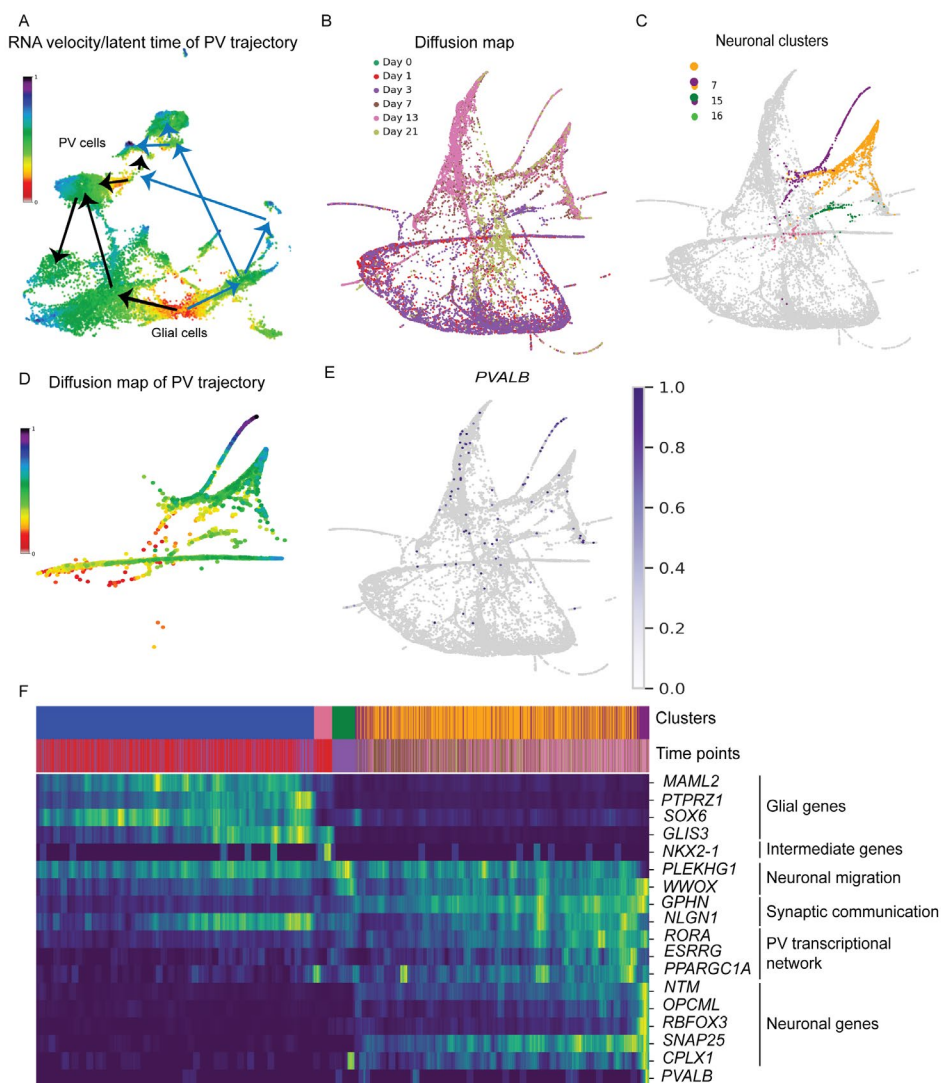


Figure 5. Trajectory of glia-to-PV reprogramming.

(A) UMAP visualizing the reprogramming trajectory based on latent time. (B) Diffusion map of all time points. (C) Visualization of neuronal clusters on diffusion map. (D) Representation of the reconstructed PV trajectory on the diffusion map, coloured by latent time. (E) Visualization of *PVALB*-expressing cells on diffusion map. (F) Heatmap showing the expression of the genes involved in the PV trajectory.

Transplanted Human iNs show long-lasting structural and functional maturation (Paper II)

Direct neuronal reprogramming of hGPCs into hiNs in mini spheres

In paper I, we demonstrated that we could obtain functional neurons *in vitro* that were similar to *bona fide* interneurons. Nonetheless, the survival and integration of transplanted human reprogrammed neurons *in vivo* are to be investigated. In this study, hGPCs were used to generate human induced neurons (hiNs) in mini spheres (Figure 6A). Importantly, these mini spheres have a smaller diameter (approximately 80 μm) compared to the 3D cultures of paper I, facilitating direct injection into the mouse brain by using standard stereotaxic set ups and increasing survival compared to single cell suspension. ADLSF combination was used to transduce hGPCs, along with a green fluorescent protein (GFP) reporter, to enable cell identification in the transplanted animals.

To confirm a successful neuronal conversion before transplantation, we examined gene and protein expression of mini spheres. RT-qPCR showed upregulation of the viral *Ascl1* gene, which is an important pioneer TF, in ADLSF-transduced mini spheres (Figure 6C). Additionally, there was an upregulation of the pan-neuronal genes *SYN1* and *MAP2* (Figure 6C). In line with this, immunostaining showed strong induction of MAP2 and TAU proteins in ADLSF group, while unconverted hGPCs maintained the expression of glial markers PDGFR α and GFAP (Figure 6D).

These data confirmed the successful generation of hiNs from hGPCs in a mini sphere 3D environment.

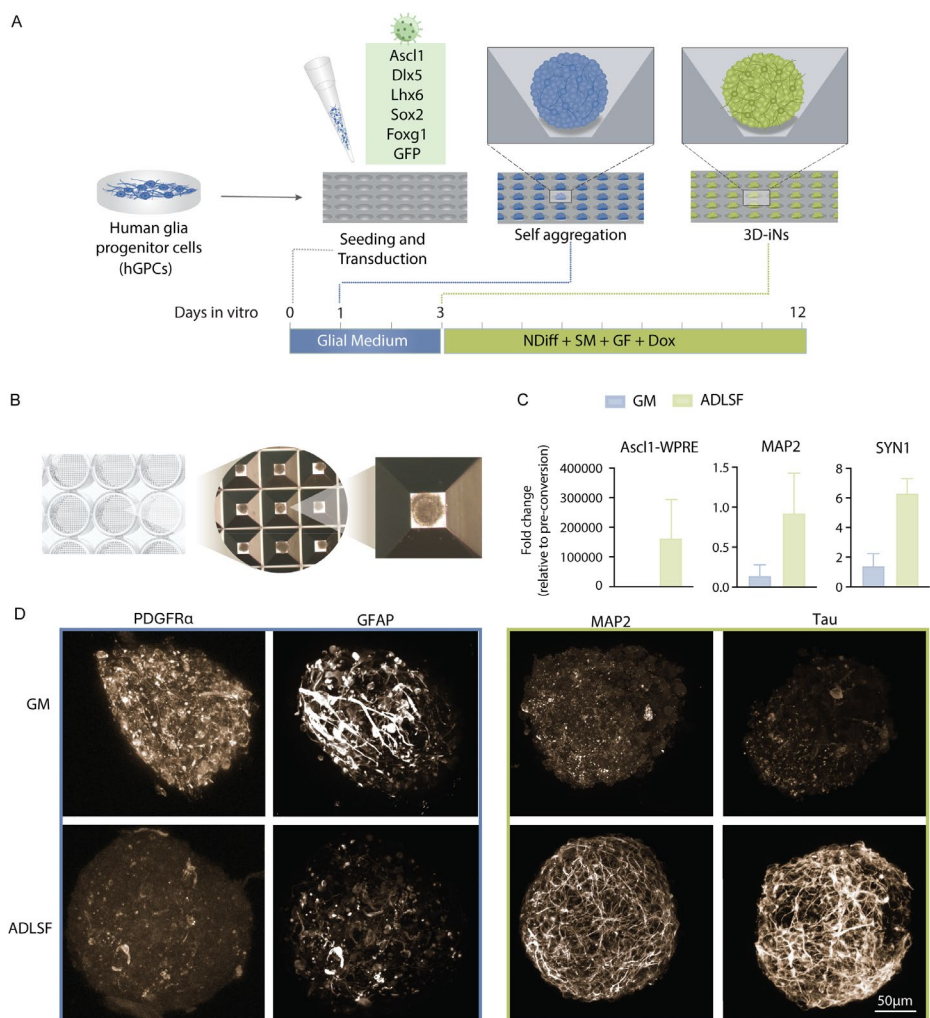


Figure 6. Neuronal reprogramming of hGPCs into hiNs in 3D mini-spheres.

(A) Schematic overview of the reprogramming process. (B) Brightfield image of mini spheres in microwells. (C) RT-qPCR analysis for *Ascl1*-WPRES, *MAP2* and *SYN1*. (D) Maximum intensity projection of immunostainings of control (GM) and ADLSF-transduced spheres with PDGFR α , GFAP, *MAP2* and *TAU*.

Long term survival, structural and functional maturation of transplanted hiNs in the mouse brain

To assess the survival and integration of hiNs into the host mouse brain, mini spheres were stereotactically injected in the PFC of NSG immunodeficient mice at postnatal day 14 (P14). Grafted mini spheres were assessed at different months post-transplantation (MPT) (Figure 7A).

Cell survival was first assessed at 1 MPT using the human-specific neural cell adhesion molecule (hNCAM) marker to detect the transplanted cells. hNCAM+ cells could be detected, which were primarily localized at the injection site and had started infiltrating the brain (Figure 7B). At this time point, cells exhibited an immature bipolar morphology and were primarily found around the graft core (zoom-in) until 3 MPT. The injection core was no longer visible at 5 and 10 MPT, likely due to cell migration and partial cell loss. The remaining hiNs continued developing complex neuronal morphology (Figure 7C).

To further examine the continuous development of their neuronal morphology over time, cells were 3D reconstructed across different time points post-transplantation. A 3D visualization clearly demonstrated a gradual structural maturation of the hiNs, as seen by constant development of neuronal complexity (Figure 7D).

To explore the functional maturation of hiNs, we performed patch-clamp electrophysiology on GFP+ transplanted cells (Figure 7E). hiNs exhibited gradual functional maturation over time, with evoking APs present at 3 and 5 MPT (Figure 7F).

Overall, these data demonstrated long-term survival and integration into the host mouse brain, allowing hiNs to progressively mature both structurally and functionally.

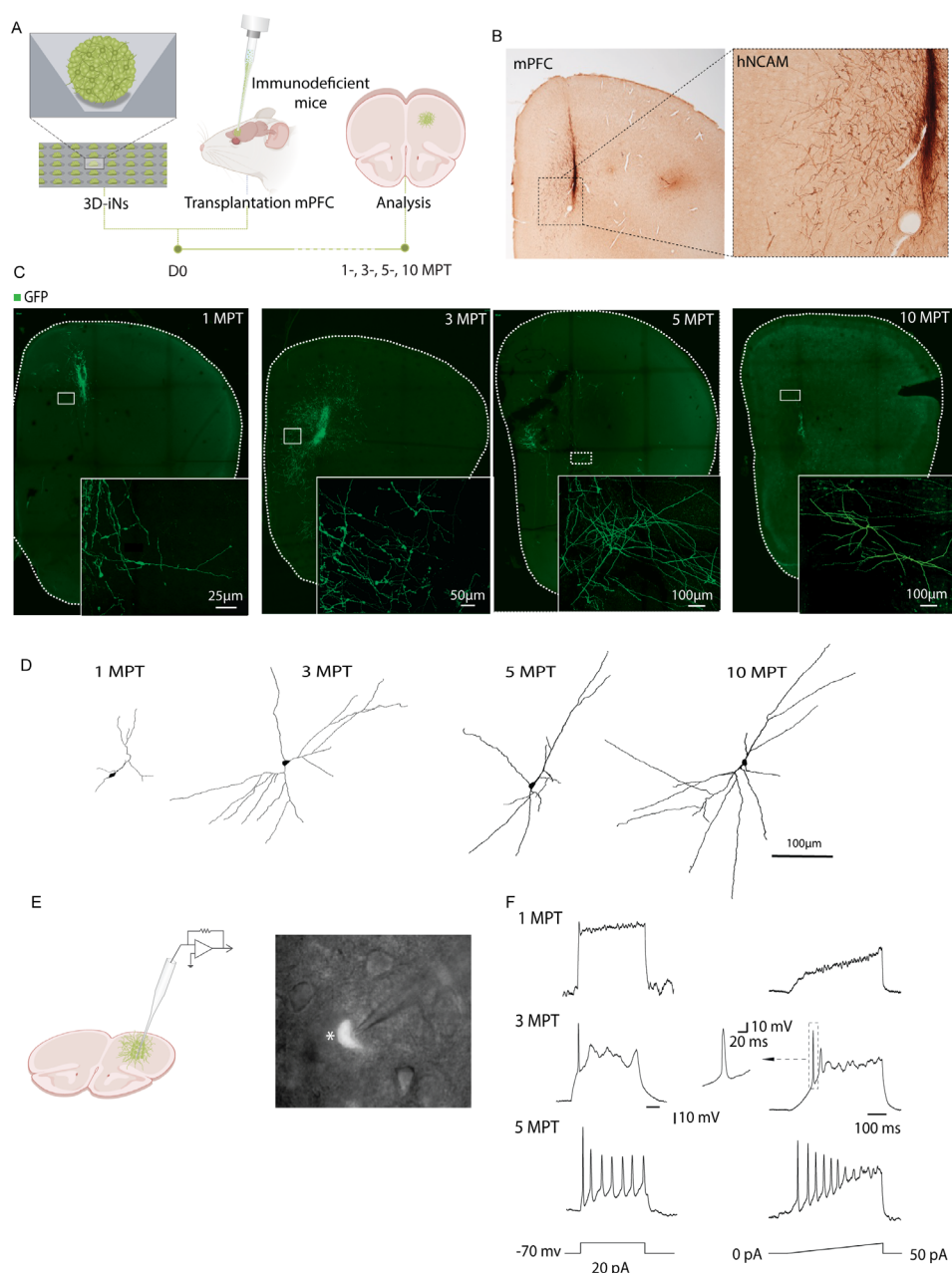


Figure 7. Long survival and maturation of transplanted hiNs into mouse PFC.

(A) Schematic overview of transplantation process. (B) Immunostaining of sectioned mouse PFC with human NCAM (hNCAM). (C) Immunostainings of transplanted hiNs with GFP at different time points post-transplantation. (D) Reconstruction of transplanted hiNs across different time points post-transplantation. (E) Schematic overview and photo of transplanted hiNs during electrophysiology recordings. (F) Representative traces of evoked action potentials upon current injection of transplanted hiNs across time points post-transplantation. PFC=prefrontal cortex.

Derivation of human subclass-specific SST interneurons in 3D co-cultures (Paper III)

Generation and maturation of MGE-like progenitors up to 100 DIV

Several studies have described successful generation of high-purity MGE progenitors (Bershteyn et al., 2023; Birey et al., 2017; Kim et al., 2014; Y. Liu et al., 2013; Maroof et al., 2013; Ni et al., 2019; Nicholas et al., 2013; Xiang et al., 2017). However, these protocols demand extended maturation period. Furthermore, there is lack of robust *in vitro* models for generation of subclass-specific SST interneurons. In this study, MGE-like progenitors and interneurons were generated according to (Nicholas et al., 2013) with minor modifications. Interneurons were co-cultured in 3D spheroids, enabling long-term culture and better synaptic communication, in the following combinations: with human glial progenitor cells (hIN+hGPC) or mouse astrocytes (hIN+mAST) or alone as interneurons (hIN). 3D co-cultures were kept for up to 100 DIV (Figure 8A).

First, we analysed gene expression levels with RT-qPCR. This demonstrated early upregulation of the forebrain marker *FOXP1* and the MGE marker *NKX2-1*, and their expression was retained throughout culture time, indicating a successful acquisition and maintenance of ventral forebrain fate. At 50 DIV, we observed upregulation of other MGE-associated transcription factors, such as *ASCL1* and *LHX6*, regardless of co-culture condition. Interestingly, *DLX5* showed higher upregulation in the hIN+hGPCs condition (Figure 8B), potentially due to expression of this marker from hGPCs (Figure 8B).

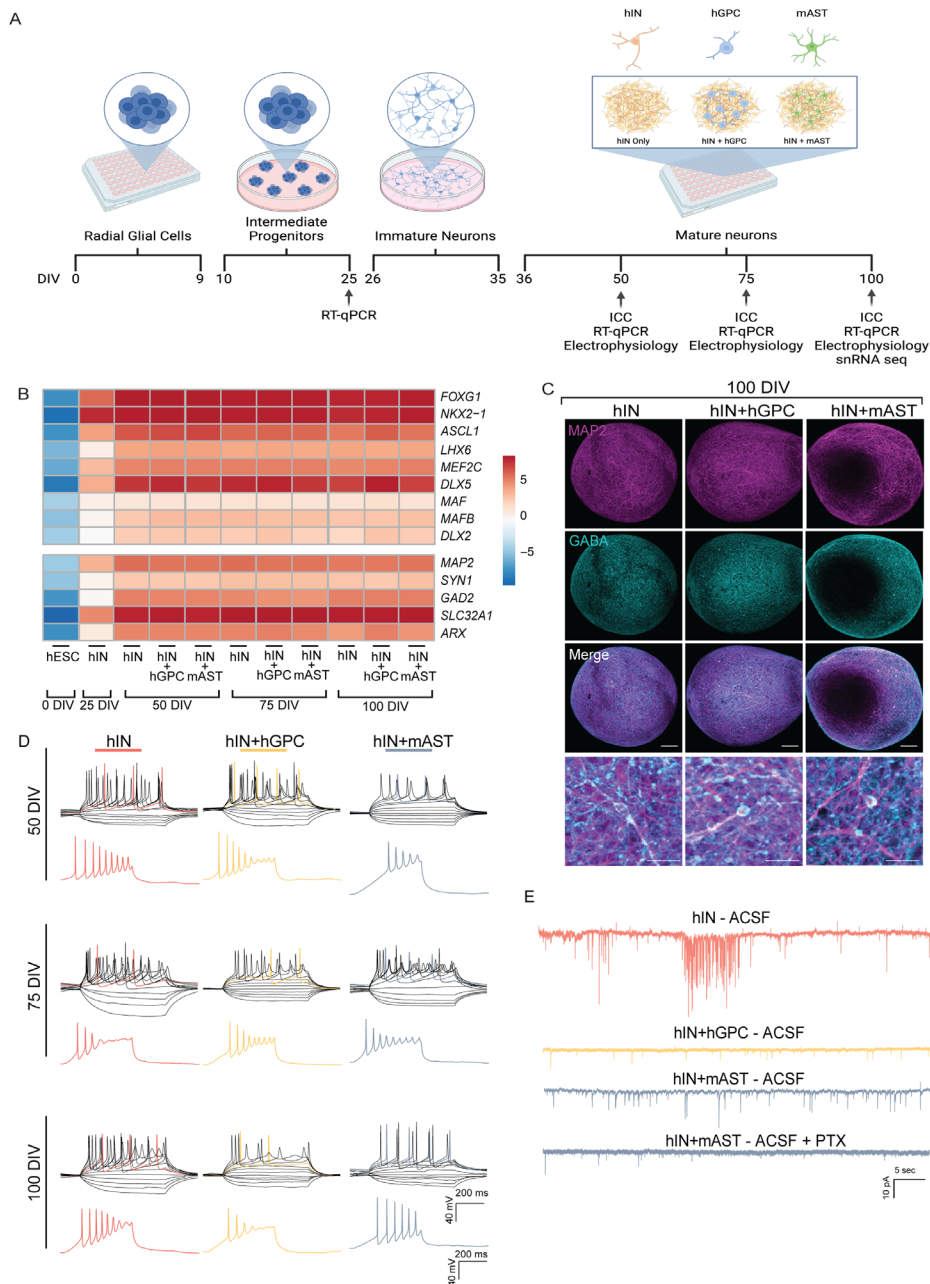


Figure 8. Differentiation of hESCs into GABAergic interneurons in a 3D co-culture system.

(A) Schematic overview of differentiation. (B) Heatmap showing the expression levels of interneuron and pan-neuronal markers across time points and conditions. (C) Maximum intensity projections of immunostainings of all groups with MAP2 and GABA at 100 DIV. Scale bar: 100 μ m. (D) Representative traces of evoked action potentials in the different culture conditions at 100 DIV. (E) Sample traces of postsynaptic activity blocked with 100 μ M PTX at 100 DIV for hIN+mAST condition. DIV=Days *in vitro*.

Upregulation of mature neuronal genes, such as *MAP2* and *SYN1*, were detected already at 50 DIV. Also, the mature GABAergic markers *GAD2* and *SLC32A1* (encoding the vesicular GABA transporter VGAT), were significantly upregulated (Figure 8B). Immunocytochemistry further confirmed our findings on transcriptional level. Staining for MAP2 and GABA at 100 DIV showed widespread distribution of MAP2+/GABA+ cells which exhibited typical neuronal morphology (Figure 8C), verifying the presence of GABAergic interneurons on protein level as well.

Next, we explored the functional maturation of interneurons in the 3D cultures over time. Application of gradually increased current injections showed the ability of the cells to fire multiple APs. The hIN+mAST group showed more mature firing at 100 DIV related to the other culture conditions (Figure 8D). Moreover, the postsynaptic currents were entirely blocked with the addition of picrotoxin (PTX), a GABA_AR antagonist (Figure 8E), confirming the GABAergic nature and efficient synaptic communication.

Taken together, these data showed that ESCs can successfully differentiate into functional GABAergic interneurons in 3D co-culture systems, which could be maintained up to 100 DIV.

Maturation of MGE-derived interneurons to SST subtype with subclass specification

Next, we examined the presence of mature interneuron subtypes generated by MGE-like precursors. Firstly, we investigated their gene expression profile with RT-qPCR. We could detect significant upregulation of the neuropeptide *SST* at 50 DIV, with constant expression until 100 DIV. Moreover, we could detect upregulation in *NPY* and *CALB2*, increasing from 50 DIV until 100 DIV (Figure 9A). Next, we investigated the presence of SST subclass genes. RT-qPCR analysis showed significant upregulation of upper layer marker *CBLN4* at 50 DIV, especially in co-culture conditions and expression was retained up to 100 DIV (Figure 9A). *TH*, a lower layer marker, was also significantly upregulated already from 25 DIV and increased at 50 DIV, particularly in co-culture conditions. (Figure 9A).

To further characterize the SST population and dissect cell type composition in the 3D co-cultures, we performed snRNA-seq at 100 DIV for all three culture groups (Figure 9B). Integration of the data revealed similar transcriptomic profile and cell type composition among the conditions. The one exception was a separate cluster derived from the hIN+hGPC group (Figure 9C, D).

Further sub clustering and integration of the SST+ cells from all groups showed three discrete subgroups (Figure 9E). The *SST*-expressing cells expressed *TH*, suggesting the presence of this subclass (Figure 9F). Genes characteristic of Martinotti cells and long-projection neurons were expressed in the SST cluster and

showed uniform expression (Figure 9G, I). However, non-Martinotti genes were preferentially expressed in the SST1 cluster (Figure 9H).

To confirm SST expression on protein level, we performed immunocytochemistry and found widespread SST protein expression at 100 DIV in all groups (Figure 9J). Remarkably, we detected co-localization of SST with TH or calretinin (CR, encoded by *CALB2* gene, Figure 9K) suggesting the presence of these SST subclasses on protein level too.

In conclusion, these data suggested a rapid maturation into SST interneurons with subclass characteristics including all the three main subclasses (Martinotti, non-Martinotti and long-projecting) in the 3D co-culture system, regardless of the culturing condition.

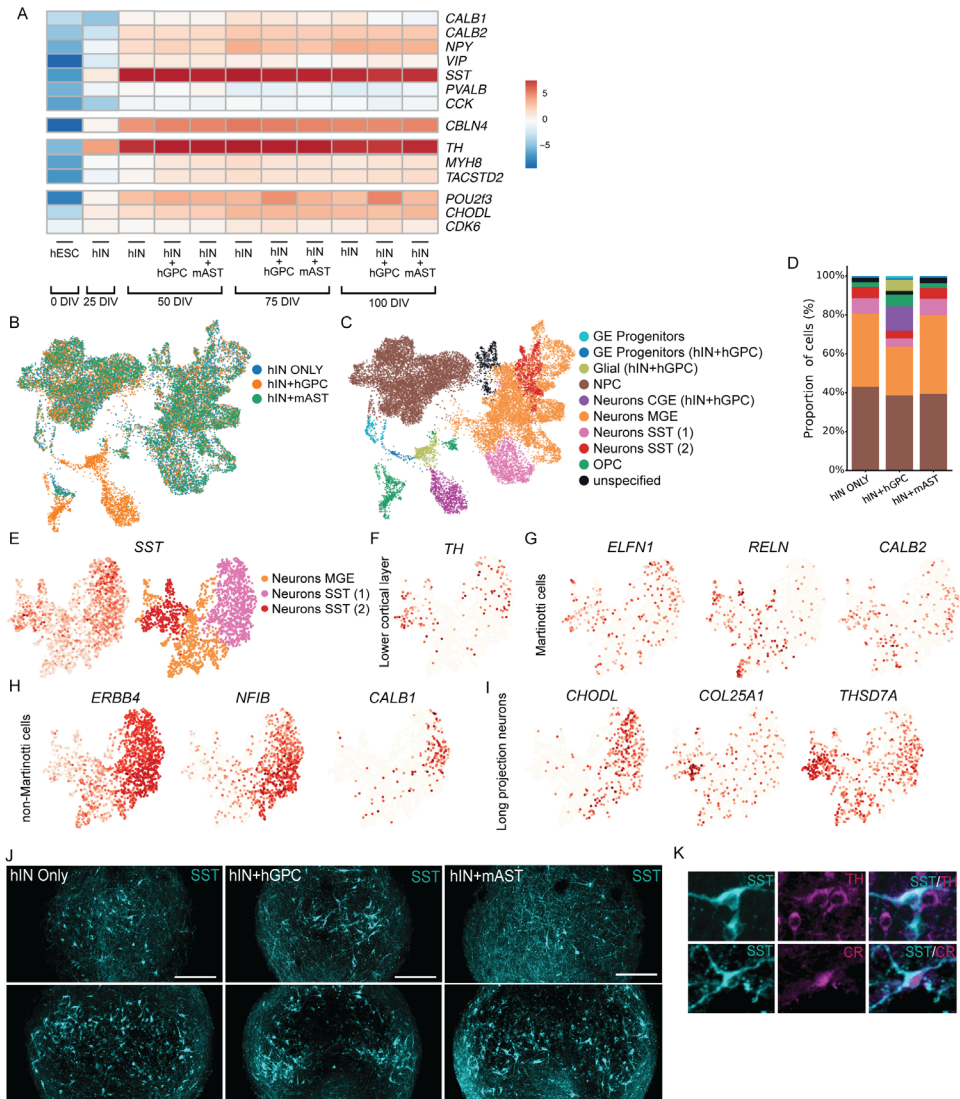


Figure 9. Generation of subclass-specific SST interneurons in the 3D co-system

(A) Heatmap showing the expression levels of subtype-specific and SST subclass-specific markers across time points and conditions. (B) UMAP plot visualizing the cells at different culture conditions at 100 DIV. (C) UMAP plot visualizing the cellular composition at different culture conditions at 100 DIV. (D) Bar plot showing the proportion of cells in each culture condition. (E) UMAP plot visualizing SST-expressing cells and their cellular composition. (F, G, H, I) UMAP plots visualizing the expression of Lower cortical layer (F), Martinotti (G), non-Martinotti (H), and long projection neuron (I) markers on SST-expressing cells. (J) Maximum intensity projection of immunostainings of hIN, hIN+hGPCs, hIN+mAST with SST at 100 DIV. Scale bar: 100 μ m. (K) Maximum intensity projection of immunostainings of with SST, TH and CR at 100 DIV. Scale bar: 10 μ m. DIV=Days *in vitro*. hIN=human interneurons, hGPCs=human Glial Progenitor Cells, mAST=mouse Astrocytes.

Integration and maturation of MGE-derived interneurons in human forebrain organoids

Next, we sought to explore if SST interneurons could survive within a human cortical system and if their subtype and subclass specificity was influenced by a more complex environment. Therefore, MGE-like progenitors marked with GFP were injected at 35 DIV into human forebrain organoids of the same age (35 days) (Figure 10A). Imaging of the endogenous GFP in organoids at 15-20 days post-injection (DPI) showed migration of the MGE interneurons toward the periphery, exhibiting complex morphology and long projections. At 40 DPI, injected cells showed a more distinct peripheral localization (Figure 10B). Immunocytochemistry with GFP, GABA and SST at 65 DPI demonstrated co-localization of GFP with GABA or GFP and SST (Figure 10C) and sometimes both proteins, (SST and GABA) (Figure 10D).

To dissect the cell type composition of the organoid-injected cells, we performed snRNA-seq at 65 DPI (corresponding to 100 DIV) on FACS-sorted GFP⁺ nuclei. An integrated UMAP of the organoid-injected cells versus the spheroid-derived cells (from all three groups) demonstrated similar transcriptomic profile and cell type composition (Figure 10E). A comparative analysis showed increased proportion of MGE and SST2 cells in organoid-injected cells (Figure 10F, G). UMAP with integrated *SST* cells from injected and spheroid-derived cells and comparative proportion analysis showed that *SST*-expressing cells in organoids mainly came from SST2 and MGE groups (Figure 10H, I). *SST*-expressing cells showed similar proportions of Martinotti and long-projecting neuron-related genes between spheroid and organoid, but lower expression of non-Martinotti genes (especially for the *ERBB4* and *NF1B*) in the organoid. Furthermore, there was a modest increase *TH* and *CDK6* co-expressing cells in organoid-derived *SST* cells (Figure 10J).

Collectively, these data demonstrated that the SST interneurons successfully survived and integrated into the human forebrain environment for up to 65 days (corresponding to 100 DIV), maintaining their SST expression and their subclass characteristics.

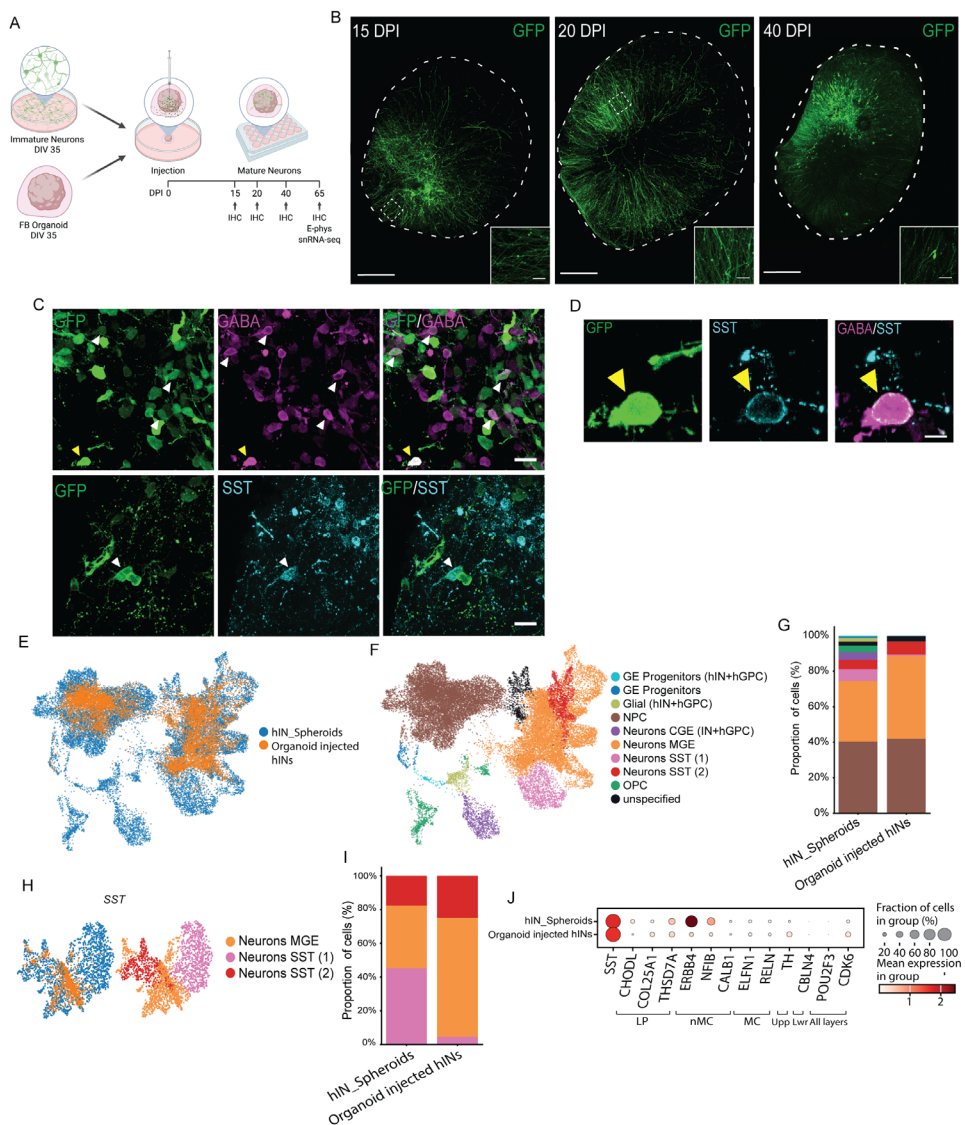


Figure 10. Survival and integration of injected MGE interneurons in human forebrain organoids.

(A) Schematic overview of cells' injection into human forebrain organoids. (B) Maximum intensity projection of injected GFP-expressing neurons in the organoids across different time points post-injection. Scale bar: 500 μ m (C) Maximum intensity projection of immunostainings of cryosectioned injected organoids with GFP, GABA and SST at 65 DIV. Scale bar: 20 μ m (D) Maximum intensity projection of immunostainings of cryosectioned injected organoids, with GFP, GABA and SST at 65 DPI. Scale bar: 50 μ m (E) UMAP visualizing integrated cells from spheroids and organoid-injected hINs at 100 DIV and 65 DPI respectively. (F) UMAP showing the cellular composition of integrated cells. (G) Barplot showing the proportions between cells from spheroids and organoid-injected hINs. (H) UMAP visualizing the integrated SST-expressing cells from spheroids and organoid-injected hINs. (I) Bar plot showing the proportion of cells between SST-expressing cells from spheroids and organoid-injected hINs. (J) Dotplot showing the expression of subclass-specific markers between SST-expressing cells from spheroids and organoid-injected hINs. DIV=Days *in vitro*.

Patient hiPSC-derived interneurons from a schizophrenia parent-case trio reveal GABAergic signalling alterations (Paper IV)

Successful derivation of GABAergic interneurons from schizophrenia parent-case hiPSCs

Some hiPSC studies on SCZ have demonstrated alterations in neuronal maturation, and morphological complexity (Dubonyte et al., 2022). However, these deficits have only been assessed on neuronal precursors or broader interneuron subtypes. There are few studies assessing SCZ-related pathologies on subtype-specific iPSC-derived GABAergic interneurons. In this study, we generated an interneuron-based model of SCZ utilizing the protocol we established at Paper III (Bruzelius et al., 2025). A parent-case trio, consisting of unaffected mother-CTR1, unaffected father-CTR2 and a son affected with schizophrenia-SCZ was used for this study (Hathy et al., 2020; Hathy et al., 2021) (Figure 11A).

Brightfield imaging was used to monitor neuronal differentiation and observe potential differences among the three lines. At 9 DIV, embryoid bodies were uniform and similar in size (Figure 11B) and at 17 DIV, neuronal rosettes were formed, where progenitors migrated uniformly from the core of the attached embryoid bodies (Figure 11B). At 33 DIV, neuronal-looking cells could be detected in all three lines (Figure 11B) and cells were thereafter replated onto human astrocytes (Figure 11B) generated from RC17-derived hGPCs.

Next, we assessed gene expression levels of important forebrain interneuron markers using RT-qPCR to confirm their identity. At 25 DIV, there was strong upregulation of *FOXG1* (forebrain marker), *ASCL1*, *DLX5*, *MEF2C* (pan-interneuron markers) *NKX2-1* and *LHX6* (MGE-specific interneuron markers), suggesting successful acquisition of MGE fate across the lines (Figure 11C). Additionally, we examined the expression mature neuronal genes. RT-qPCR showed upregulation of mature neuronal markers, *MAP2* and *SYN1* and the pan GABAergic marker *SLC32A1* (*VGAT*) at 35 DIV. We could also detect an upregulation of the interneuron subtype-specific marker *SST* (Figure 11D). While this was seen for all three lines, SCZ line showed the lowest upregulation of *SST*. These results demonstrate expression of mature interneurons genes in all three lines and upregulation of *SST* expression.

The neuronal cultures were further assessed at 35 DIV with immunocytochemistry to profile their protein expression. We observed cells positive for both MAP2 and GABA in all three lines, confirming the presence of GABAergic interneurons (Figure 11E). Quantification of MAP2+ cells over DAPI and GABA+ cells over MAP2+ cells showed comparable proportions across the three lines (Figure 11F).

We could also detect protein expression of SST and MAP2 (Figure 11E), with similar percentages of %SST+/MAP2 cells among the lines (Figure 11F).

Overall, these data demonstrated a successful induction of MGE fate in all lines and rapid differentiation into GABAergic interneurons including SST subtype at 35 DIV.

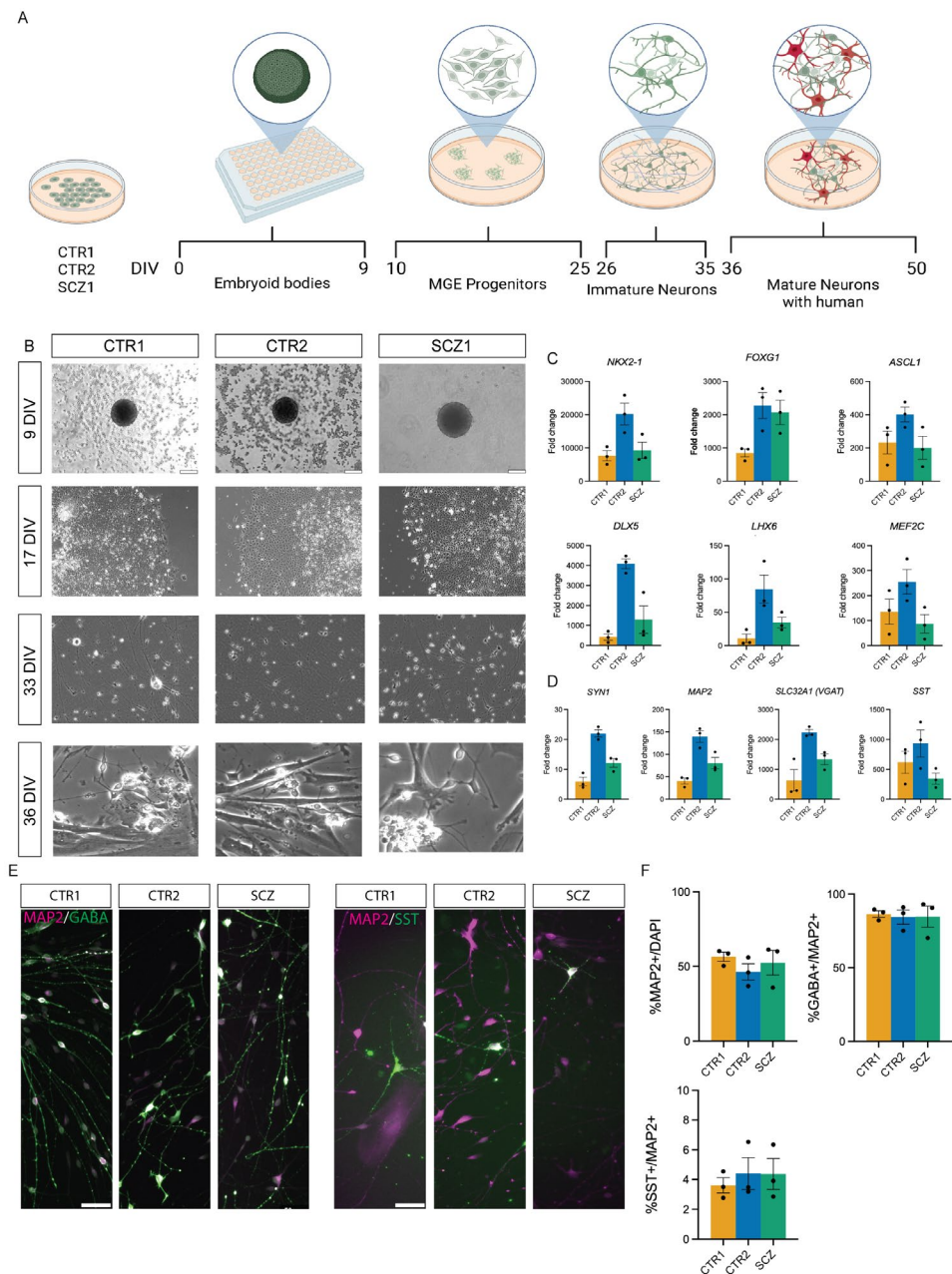


Figure 11. Successful generation of MGE-like progenitors and interneurons for all hiPSC lines. (A) Schematic overview of differentiation process. (B) Brightfield photos of the cells at different stages of differentiation in all three lines. (C) RT-qPCR analysis of the expression of interneuron markers at 25 DIV across the three lines. (D) RT-qPCR analysis of the expression of mature neuronal markers at 35 DIV across the three lines. (E) Immunostainings for MAP2, GABA and SST at 35 DIV in all three lines. (F) Quantification analysis of neuronal cultures at 35 DIV. Scale bar: 50 μ m. DIV=Days *in vitro*.

Patient-derived interneurons demonstrate functional impairments and GABA response alterations.

To explore the functional properties of the interneurons, we performed whole-cell patch clamp electrophysiology recordings of hiPSC-derived neurons co-cultured with human astrocytes at 50-57 DIV. Spontaneous activity was assessed in current clamp. Neurons in both control groups (CTR1 and CTR2) displayed spontaneous action potential (AP) firing, while most SCZ neurons did not (Figure 12A). In line with this, the fraction of spontaneously active neurons was lower in the SCZ group (approximately 26%) compared to CTR1 (62%) and CTR2 (50%) (Figure 12B).

To investigate intrinsic excitability, AP firing was evoked by stepwise current injections. Control neurons typically generated repetitive AP firing upon current injection, whereas SCZ neurons most often generated only a single AP (Figure 12C). Additionally, SCZ neurons exhibited reduced AP amplitude and reduced maximum number of APs compared to controls (Figure 12D). Together, this data revealed reduced intrinsic excitability and impaired firing capacity in SCZ interneurons.

Next, we explored GABA_A receptor-mediated signalling using brief local puffs of GABA to the soma and the proximal dendrites (Figure 12E). CTR1 and CTR2 neurons displayed robust inward currents in response to GABA puffs (Figure 12F, G). On the contrary, most SCZ neurons showed no detectable current under the same conditions (Figure 12F, G). Indeed, all tested neurons in CTR1 group and 75% of neurons in CTR2 group responded to GABA, while only 11% of SCZ neurons responded (Figure 12H).

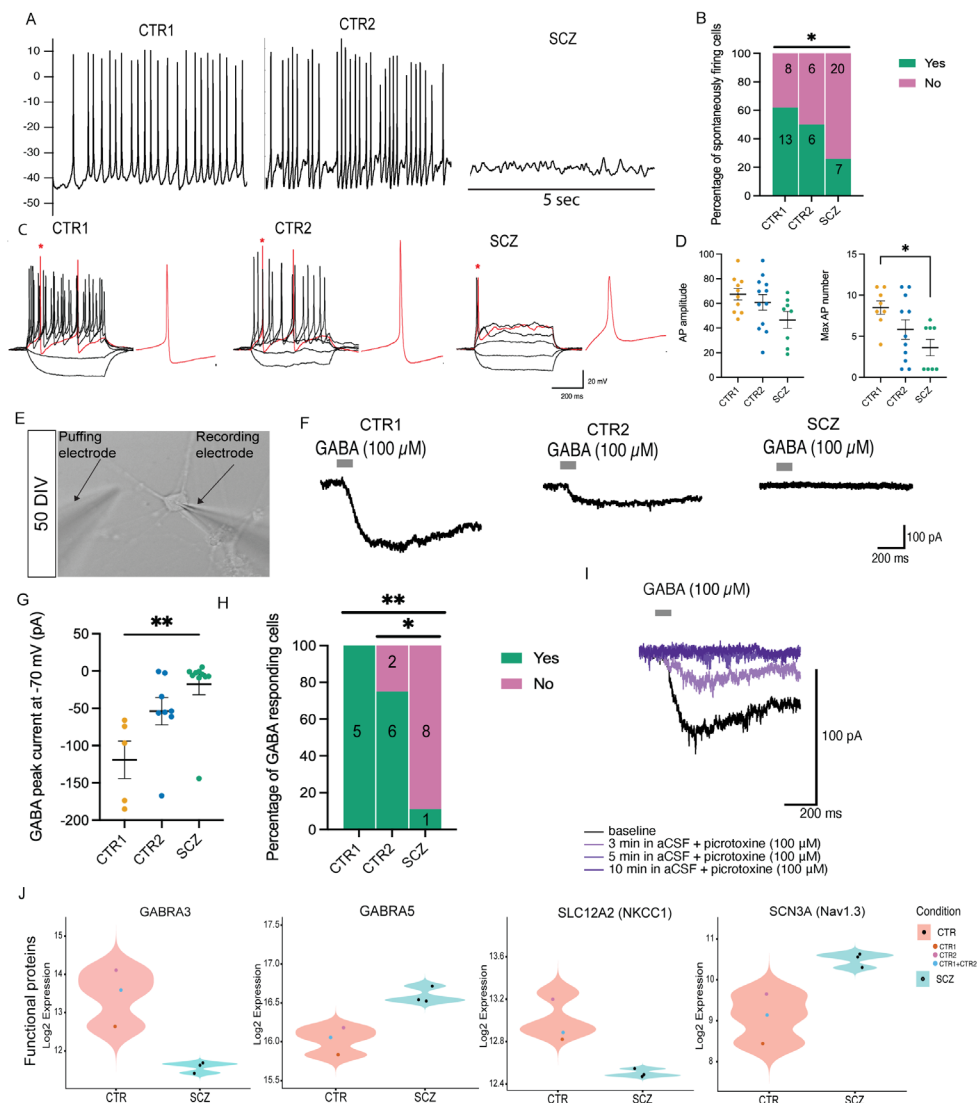


Figure 12. Functional impairment and GABAergic remodelling in patient hiPSCs-derived neurons at 50 DIV.

(A) Spontaneous firing of neurons for the three lines at 50 DIV. (B) Fraction of cells spontaneously firing at 50 DIV (Fisher's exact test, $p < 0.05$ *). (C) Representative traces of action potentials upon current injection for across the three lines at 50 DIV. (D) Quantification of AP amplitude and max AP number for all the three lines at 50 DIV ($n = 10$ cells for CTR1, $n = 12$ for CTR2, $n = 8$ for SCZ, $p < 0.05$ *). (E) Brightfield image of neuron with the puffing electrode. (F) Representative samples of neurons responding to GABA for all three lines at 50 DIV. (G) GABA peak current at -70 mV for the three lines at 50 DIV ($n = 5$ cells for CTR1, $n = 8$ for CTR2, $n = 9$ for SCZ). (H) Fraction of cells responding to GABA across the line at 50 DIV (Fisher's exact test, $p < 0.05$ *, $p < 0.01$ **). (I) Current after application of picrotoxin in the bath. (J) Violin plots visualizing the differences in protein expression of GABRA3, GABRA5, SLC212A2 and SCN3A between CTR and SCZ group. DIV=Days *in vitro*.

To further dissect the mechanisms behind the functional analysis results, we proceeded with proteomics experiment. The groups included in this analysis were patient and controls (CTR1, CTR2 and CTR3 that consisted of a pool of CTR1 and 2). We explored the expression of proteins that were relevant to GABAergic function. Interestingly, the GABA_A receptor subunit GABRA3 showed a lower expression in SCZ compared to CTR group, (Fold change 3.66, p value<0.05, adjusted p value=0.09), while the GABA_A receptor subunit GABRA5 showed higher expression in SCZ (Fold change 1.48, p value<0.05, adjusted p value=0.07). This suggested altered GABAergic signalling in patient derived neurons. SLC12A2 (or NKCC1), showed modest reduced levels (Fold change 1.39, p value <0.05, adjusted p value<0.05). Yet, the voltage-gated sodium channel SCN3A or Nav1.3, was upregulated in SCZ (Fold change 2.64, p value <0.05, adjusted p value=0.12 although the adjusted p value was more than 0.05) (Figure 12J).

Collectively, functional analysis and proteomics data suggested impaired intrinsic excitability and remodelling of GABAergic signalling in hiPSC-derived neurons at 50 DIV, accompanied by significant protein alteration in GABAergic receptor subunits, ion transporters and voltage-gated ion channels.

Discussion and future perspectives

Cortical interneurons are crucial for proper brain function and are involved in processes, such as sensory information processing, motor output control, and mediation of higher-order cognitive functions, such as planning and decision-making (Carlen, 2017; Kepecs & Fishell, 2014). PV and SST interneurons are lost or dysfunctional in several diseases, such as schizophrenia, epilepsy and autism spectrum disorder (Contractor et al., 2021; Leitch, 2024; Marin, 2012, 2024). Advances in cell culture and transcriptomic techniques have allowed us to dissect their developmental timeline and mimic their development in the lab.

In this thesis, we generate human PV and SST interneurons through glial reprogramming for cell replacement and stem cell differentiation for disease modelling, respectively.

Cell transplantation of GABAergic interneurons has been explored as therapeutic strategy for neurodevelopmental disorders and has been tried on preclinical models of neurodevelopmental disorders (Li et al., 2022; Southwell et al., 2014). There is currently a clinical trial ongoing testing cell therapy for epilepsy (NTE001 and NTE002), where researchers generate stem-cell derived MGE interneurons that appear to restore brain function in mouse epileptic models and acquire subtype specificity, including both PV and SST interneurons, in the mouse brain (Bershteyn et al., 2023; Bershteyn et al., 2025).

In this thesis, we propose an alternative strategy for cell replacement of subtype-specific interneurons. Direct reprogramming bypasses pluripotency step, suggesting a faster way to generate the cells of interest. Furthermore, the application can be transferred *in vivo*, where cells can be converted *in situ*, taking advantage of an *in vivo* microenvironment (Pereira et al., 2019; Vignoles et al., 2019). This approach avoids technical complications of allogenic transplantation, such as *ex vivo* expansion and potential immune rejection.

In **Paper I**, we pioneer in demonstrating direct reprogramming of hGPCs to chandelier PV interneurons. We were able to demonstrate that we get PV gene and protein expression already at 1 week of reprogramming. This is the fastest that has been reported so far in the literature, since most of the studies using stem cell differentiation report much longer timelines (Nicholas et al., 2013), taking also into consideration that PV immunoreactivity comes late in human brain development making it difficult to mimic physiological brain development in the labs (Marin,

2025). One major question that this study answered was whether the reprogrammed neurons are *bona fide* with endogenous ones, given that this has been a debate in the reprogramming field. We are the first to demonstrate that these reprogrammed cells are similar to their endogenous counterpart on transcriptional level, by comparing with post-mortem human adult cortical interneurons. Furthermore, we demonstrated that on transcriptional level, these PV interneurons are of chandelier subclass, which has not been extensively reported in other interneuron studies. Moreover, we also showed that reprogrammed interneurons expressed genes necessary for their synaptic machinery. Classical pharmacology experiments suggested the presence of functional GABAergic communication. However, we could not detect fast-spiking activity or other types of synaptic communication, such as glutamatergic. This may be due the lack of other inputs e.g. excitatory signalling, as well as timing in the culture or media composition, which may not support their high metabolic demands. A future strategy would be to co-culture these interneurons with other types of neurons, either in conventional 2D cultures or in assembloid systems, or further refine the culture time and conditions. It is important that reprogrammed interneurons exhibit proper functional signalling to restore excitation/inhibition balance in the diseased brain.

To tackle this, in **Paper II** we transplanted the neurons generated from paper I and demonstrated that they can last up to 10 months in the mouse PFC, accompanied by functional and structural maturation. This is the first study showing that transplanted hiNs, can survive for so long. Furthermore, this is the first study to reconstruct transplanted hiNs and demonstrate their impressive neuronal structural maturation over time in the mouse brain. It has been previously shown that transplantation of reprogrammed neurons in 3D cultures enables survival and maturation (Kajtez et al., 2025). Nonetheless, cells remained in a juvenile state and we could not detect mature neuronal activity. Also, transplanted cells seemed to lose their PV protein expression. We hypothesize that this could be due to the fragility of mature neurons (something also observed in Paper I) or due to some plasticity mechanisms (Winkel et al., 2021). hiNs may need further support, such as co-delivery with growth factors (Wang et al., 2023), exercise (Moriarty et al., 2025) and electrical stimulation (Wang et al., 2025).

Direct reprogramming is currently explored in a clinical setting. There is strong proof-of-concept harnessing direct reprogramming for cancer immunotherapy, where cancer cells are *in situ* reprogrammed into dendritic cells (Asci et al., 2024). Additionally, there are clinical trials ongoing where they employ *in situ* reprogramming of astrocytes into mature neurons as therapeutic strategy for Alzheimer's, stroke (NXL-001) and glioblastoma (NKL-004).

Despite the advantages of direct reprogramming, there are few things to take into consideration for neuronal direct reprogramming to move forward into clinical applications. One very important parameter is the vector design and virus capsid engineering to just target the starting cell type of interest and avoid off-target

problems. Secondly, to find the right cocktail of TFs to generate the proper neuronal type one wants to replace and extensively characterize the newly generated neurons on transcriptomic, protein and functional level. Another important thing is the right starting population. Even for *in vitro* proof-on-concept studies, it is critical to use starting cell populations which are *bona fide* to their endogenous counterpart. In our case, the hGPCs we utilize closely reassemble cortical *bona fide* OPCs. Finally, which brain location to choose for conversion. Unlike Parkinson's and epilepsy, where the pathology is more localized in the brain, location might a problem for diseases with more diffuse pathology, such as schizophrenia, where deficits have been reported in cortex, hippocampus and other brain regions (Adamu et al., 2023).

Given interneurons' involvement in neurodevelopmental and neuropsychiatric disorders, research has focused on generating reliable and robust disease models from patient iPSCs to dissect interneuron-related disease mechanisms. iPSC platforms have been commercialized and widely used for drug screening purposes. There have been several studies generating interneuron-based disease models for disorders, such as schizophrenia and epilepsy (reviewed in (Dubonyte et al., 2022) and (Sterlini et al., 2020)), but very few studies managed to generate human subtype-specific interneurons *in vitro* from patient iPSCs.

In **paper III**, we utilize 3D co-culturing with other glial cells to generate SST interneurons from ESCs with subclass characteristics. This is the first study reporting derivation of human stem cell-derived SST interneurons with subclass characteristics. Given that SST interneurons are the most diverse interneuron subtype, studies have focused on elucidating the diversity of SST subclasses (Fisher et al., 2024; Wu et al., 2023). Notably, there are studies using mouse models and post-mortem human brain samples showing that specific subclasses of SST are involved in the pathology of neuropsychiatric disorders (Asenjo-Martinez et al., 2025; Batiuk et al., 2022; Tai et al., 2014). One limitation of the co-culturing model was the lack of other neuronal types. To tackle this, we transplanted ESC-derived interneurons into forebrain organoids, containing glutamatergic neurons, where interneurons successfully survived, integrated into the host circuitry and maintained the expression of subclass-specific markers, thus creating a more complex and physiologically relevant model. In this study we did not manage to generate PV interneurons, which have been notoriously difficult to derive *in vitro*. Recent studies harnessing organoid and assembloid technology and keeping long-term cultures have successfully managed to generate PV interneurons, enabling the investigation of these cells in development and disease (Varela et al., 2025; Walsh et al., 2025).

In **paper IV**, we utilize the model developed in paper III to generate SST interneurons from a schizophrenia parent-case trio. We demonstrated that patient derived interneurons have decreased levels of *SST* mRNA, exhibited impaired functionality and altered GABAergic signalling. This is the first study showing derivation of subtype-specific interneurons from a family case, accompanied by implications of GABAergic signalling in schizophrenia-derived interneurons.

Parent-case studies offer a unique opportunity to study disease mechanisms minimizing the genetic variability and focusing of genetic variants (Flaherty et al., 2017; Hathy et al., 2020; Hathy et al., 2021) . However, these studies focus on a very small subject number and inference of the results to general population might not be possible. Furthermore, in this model there is lack of other neuronal inputs, such as glutamatergic, which could facilitate the study of other signalling cues. The importance of this study is the fact that it sheds light on GABAergic signalling alterations and deficits, which have been less studied compared to NMDA receptor signalling. These results reinforce the idea that interneuron synaptic connectivity impairments are the driver of neurodevelopmental disorders. Future studies should focus on further dissecting the GABAergic signalling in schizophrenia and implementing drug screening approaches that effectively modulate and restore signalling to mitigate disease symptoms. Building on our findings in Paper I, where we showed that it is possible to generate interneurons that express genes critical for synaptic communication and are similar to endogenous interneurons, *in situ* direct reprogramming of glial cells could be a promising strategy to generate functional interneurons *in vivo*. This strategy could enable functional restoration of GABAergic activity in the diseased brain, highlighting its potential for future therapeutic applications.

Our study and many previous iPSC disease modelling studies use the conventional 2D monolayer culture platform. These iPSC models have been widely used to study genetic diseases and more complex multifactorial diseases. 2D models have several advantages, such as derivation of homogenous population of a cell subtype of interest and the adaptability to high-throughput screening approaches. The major disadvantages of 2D models are the lack of 3D architecture and cell heterogeneity, and cell fragility over time (Levy & Pasca, 2025).

Advances in organoid and assembloid technology have allowed us to generate more complex multi-regional brain structures composed of multiple cell types, closely reassembling the endogenous structures. They can even include other cells from other embryonic lineages, such as microglia (Sabogal-Guaqueta et al., 2024) and endothelial cells forming the vasculature (Kistemaker et al., 2025). Clinical trials testing compounds initially screened on iPSC-derived cells are progressing, demonstrating the potential of human stem cell-derived models (Okano & Morimoto, 2022).

Regarding modelling of neuropsychiatric disorders, it is essential to dissect their disease mechanisms and identify potential therapeutic targets in systems with additional functional-level readouts, using multi-electrode arrays (MEA) or other advanced electrophysiology systems, which should reflect the circuit malfunctions observed in patients. On a clinical setting, these disorders are often behaviourally diagnosed and lack robust biomarkers. Therefore, *in vitro* models should recapitulate the circuit impairments to minimize false-positive findings or impartial restoration. (Levy & Pasca, 2025). 2D culture models offer the opportunity to study

a homogenous interneuron population and find certain molecular and cellular disease phenotypes. To fully elucidate the role of interneurons in neurodevelopmental and psychiatric disorders, advanced organoid and assembloid systems should be employed to model complex circuits and apply functional readouts.

Conclusions

In this thesis, we managed to generate human subtype-specific interneurons for therapeutic cell replacement and disease modelling with different strategies.

In paper I, we derived human chandelier PV interneurons via glia reprogramming. Neurons are generated as early as 7 days post transduction and PV interneurons are transcriptionally *boda fide* with their endogenous cortical counterpart. Furthermore, we unravel the glia-to-PV developmental trajectory, showing genes important for this conversion.

In paper II, we transplant neurons generated from paper I into the mouse PFC and demonstrate that they structurally mature and exhibit improved maturation over time, surviving up to 10 months post transplantation.

In paper III, we employ stem cell differentiation to derive SST interneurons in 3D co-culture with subclass characteristics, that successfully integrate into forebrain organoids and maintain their subclass characteristics, indicating that they retain their mature transcriptional profile.

In paper IV, we utilize the model established in Paper III to generate SST interneurons from a schizophrenia parent-case trio. We demonstrate that patient derived interneurons exhibited limited firing capacity and altered GABAergic signalling, which may play a role in improper excitation/inhibition balance.

This thesis covers the cellular component of cell replacement and drug discovery. Interdisciplinary efforts should be made to bring subtype-specific interneuron cell therapy and disease modelling closer to clinical applications.

References

- Adamu, M. J., Qiang, L., Nyatega, C. O., Younis, A., Kawuwa, H. B., Jabire, A. H., & Saminu, S. (2023). Unraveling the pathophysiology of schizophrenia: insights from structural magnetic resonance imaging studies. *Front Psychiatry*, 14, 1188603. <https://doi.org/10.3389/fpsyt.2023.1188603>
- Addis, R. C., Hsu, F. C., Wright, R. L., Dichter, M. A., Coulter, D. A., & Gearhart, J. D. (2011). Efficient conversion of astrocytes to functional midbrain dopaminergic neurons using a single polycistronic vector. *PLoS One*, 6(12), e28719. <https://doi.org/10.1371/journal.pone.0028719>
- Anderson, S. A., Eisenstat, D. D., Shi, L., & Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes. *Science*, 278(5337), 474-476. <https://doi.org/10.1126/science.278.5337.474>
- Angevine, J. B., Jr., & Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature*, 192, 766-768. <https://doi.org/10.1038/192766b0>
- Asic, E., Akerstrom, F., Sreekumar Nair, M., Rosa, A., Kurochkin, I., Zimmermannova, O., Catena, X., Rotankova, N., Veser, C., Rudnik, M., Ballocci, T., Scharer, T., Huang, X., de Rosa Torres, M., Renaud, E., Velasco Santiago, M., Met, O., Askmyr, D., Lindstedt, M.,...Pereira, C. F. (2024). In vivo dendritic cell reprogramming for cancer immunotherapy. *Science*, 386(6719), eadn9083. <https://doi.org/10.1126/science.adn9083>
- Asenjo-Martinez, A., Dragicevic, K., Hou, W. H., Ozsvar, A., Winther Hansen, N., Pfisterer, U., Rydbirk, R., Demharter, S., Moller, B. E., Gasthaus, J., Korshunova, I., Perrier, J. F., Capogna, M., Vasistha, N. A., & Khodosevich, K. (2025). Dysfunction of cortical GABAergic projection neurons as a major hallmark in a model of neuropsychiatric syndrome. *Neuron*, 113(19), 3204-3223 e3211. <https://doi.org/10.1016/j.neuron.2025.08.028>
- Bakken, T. E., Hodge, R. D., Miller, J. A., Yao, Z., Nguyen, T. N., Aevermann, B., Barkan, E., Bertagnolli, D., Casper, T., Dee, N., Garren, E., Goldy, J., Graybuck, L. T., Kroll, M., Lasken, R. S., Lathia, K., Parry, S., Rimorin, C., Scheuermann, R. H.,...Tasic, B. (2018). Single-nucleus and single-cell transcriptomes compared in matched cortical cell types. *PLoS One*, 13(12), e0209648. <https://doi.org/10.1371/journal.pone.0209648>
- Bartolini, G., Sanchez-Alcaniz, J. A., Osorio, C., Valiente, M., Garcia-Frigola, C., & Marin, O. (2017). Neuregulin 3 Mediates Cortical Plate Invasion and Laminar Allocation of GABAergic Interneurons. *Cell Rep*, 18(5), 1157-1170. <https://doi.org/10.1016/j.celrep.2016.12.089>

- Batiuk, M. Y., Tyler, T., Dragicevic, K., Mei, S., Rydbirk, R., Petukhov, V., Deviatiiarov, R., Sedmak, D., Frank, E., Feher, V., Habek, N., Hu, Q., Igoalkina, A., Roszik, L., Pfisterer, U., Garcia-Gonzalez, D., Petanjek, Z., Adorjan, I., Kharchenko, P. V., & Khodosevich, K. (2022). Upper cortical layer-driven network impairment in schizophrenia. *Sci Adv*, 8(41), eabn8367. <https://doi.org/10.1126/sciadv.abn8367>
- Bergen, V., Lange, M., Peidli, S., Wolf, F. A., & Theis, F. J. (2020). Generalizing RNA velocity to transient cell states through dynamical modeling. *Nat Biotechnol*, 38(12), 1408-1414. <https://doi.org/10.1038/s41587-020-0591-3>
- Bershteyn, M., Broer, S., Parekh, M., Maury, Y., Havlicek, S., Kriks, S., Fuentealba, L., Lee, S., Zhou, R., Subramanyam, G., Sezan, M., Sevilla, E. S., Blankenberger, W., Spatazza, J., Zhou, L., Nethercott, H., Traver, D., Hampel, P., Kim, H.,...Nicholas, C. R. (2023). Human pallial MGE-type GABAergic interneuron cell therapy for chronic focal epilepsy. *Cell Stem Cell*, 30(10), 1331-1350 e1311. <https://doi.org/10.1016/j.stem.2023.08.013>
- Bershteyn, M., Zhou, H., Fuentealba, L., Chen, C., Subramanyam, G., Cherkowsky, D., Sevilla, E. S., Hampel, P., Salvatierra, J., Sezan, M., Maury, Y., Havlicek, S., Kriks, S., Lee, S., Au, W., Watson, M., Kuzmenko, O., Grimmett, M. E., Vogel, A.,...Nicholas, C. R. (2025). Human stem cell-derived GABAergic interneuron development reveals early emergence of subtype diversity and gradual electrochemical maturation. *Neuron*, 113(19), 3162-3184 e3110. <https://doi.org/10.1016/j.neuron.2025.06.010>
- Birey, F., Andersen, J., Makinson, C. D., Islam, S., Wei, W., Huber, N., Fan, H. C., Metzler, K. R. C., Panagiotakos, G., Thom, N., O'Rourke, N. A., Steinmetz, L. M., Bernstein, J. A., Hallmayer, J., Huguenard, J. R., & Pasca, S. P. (2017). Assembly of functionally integrated human forebrain spheroids. *Nature*, 545(7652), 54-59. <https://doi.org/10.1038/nature22330>
- Bocchi, R., Masserdotti, G., & Gotz, M. (2022). Direct neuronal reprogramming: Fast forward from new concepts toward therapeutic approaches. *Neuron*, 110(3), 366-393. <https://doi.org/10.1016/j.neuron.2021.11.023>
- Bortone, D., & Polleux, F. (2009). KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. *Neuron*, 62(1), 53-71. <https://doi.org/10.1016/j.neuron.2009.01.034>
- Bruzelius, A., Stamouli, C. A., Holldobler, A. L., Aretio-Medina, C., Cepeda-Prado, E., Sozzi, E., Passarello, G. R., Nocera, G., Giacomoni, J., Olariu, V., & Rylander Ottosson, D. (2025). Three-dimensional co-culturing reveals human stem cell-derived somatostatin interneurons with subclass expression. *Stem Cell Reports*, 20(9), 102634. <https://doi.org/10.1016/j.stemcr.2025.102634>
- Butt, S. J., Sousa, V. H., Fuccillo, M. V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., & Fishell, G. (2008). The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. *Neuron*, 59(5), 722-732. <https://doi.org/10.1016/j.neuron.2008.07.031>
- Buzsaki, G., & Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science*, 304(5679), 1926-1929. <https://doi.org/10.1126/science.1099745>

- Caiazzo, M., Dell'Anno, M. T., Dvoretzkova, E., Lazarevic, D., Taverna, S., Leo, D., Sotnikova, T. D., Menegon, A., Roncaglia, P., Colciago, G., Russo, G., Carninci, P., Pezzoli, G., Gainetdinov, R. R., Gustincich, S., Dityatev, A., & Broccoli, V. (2011). Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature*, 476(7359), 224-227. <https://doi.org/10.1038/nature10284>
- Carlen, M. (2017). What constitutes the prefrontal cortex? *Science*, 358(6362), 478-482. <https://doi.org/10.1126/science.aan8868>
- Casarosa, S., Fode, C., & Guillemot, F. (1999). Mash1 regulates neurogenesis in the ventral telencephalon. *Development*, 126(3), 525-534. <https://doi.org/10.1242/dev.126.3.525>
- Cerneckis, J., Cai, H., & Shi, Y. (2024). Induced pluripotent stem cells (iPSCs): molecular mechanisms of induction and applications. *Signal Transduct Target Ther*, 9(1), 112. <https://doi.org/10.1038/s41392-024-01809-0>
- Chattopadhyaya, B., Di Cristo, G., Wu, C. Z., Knott, G., Kuhlman, S., Fu, Y., Palmiter, R. D., & Huang, Z. J. (2007). GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual cortex. *Neuron*, 54(6), 889-903. <https://doi.org/10.1016/j.neuron.2007.05.015>
- Chen, X., Birey, F., Li, M. Y., Revah, O., Levy, R., Thete, M. V., Reis, N., Kaganovsky, K., Onesto, M., Sakai, N., Hudacova, Z., Hao, J., Meng, X., Nishino, S., Huguenard, J., & Pasca, S. P. (2024). Antisense oligonucleotide therapeutic approach for Timothy syndrome. *Nature*, 628(8009), 818-825. <https://doi.org/10.1038/s41586-024-07310-6>
- Chouchane, M., Melo de Farias, A. R., Moura, D. M. S., Hilscher, M. M., Schroeder, T., Leao, R. N., & Costa, M. R. (2017). Lineage Reprogramming of Astroglial Cells from Different Origins into Distinct Neuronal Subtypes. *Stem Cell Reports*, 9(1), 162-176. <https://doi.org/10.1016/j.stemcr.2017.05.009>
- Ciceri, G., Dehorter, N., Sols, I., Huang, Z. J., Maravall, M., & Marin, O. (2013). Lineage-specific laminar organization of cortical GABAergic interneurons. *Nat Neurosci*, 16(9), 1199-1210. <https://doi.org/10.1038/nn.3485>
- Cobos, I., Calcagnotto, M. E., Vilaythong, A. J., Thwin, M. T., Noebels, J. L., Baraban, S. C., & Rubenstein, J. L. (2005). Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat Neurosci*, 8(8), 1059-1068. <https://doi.org/10.1038/nn1499>
- Colasante, G., Lignani, G., Rubio, A., Medrihan, L., Yekhlief, L., Sessa, A., Massimino, L., Giannelli, S. G., Sacchetti, S., Caiazzo, M., Leo, D., Alexopoulou, D., Dell'Anno, M. T., Ciabatti, E., Orlando, M., Studer, M., Dahl, A., Gainetdinov, R. R., Taverna, S.,...Broccoli, V. (2015). Rapid Conversion of Fibroblasts into Functional Forebrain GABAergic Interneurons by Direct Genetic Reprogramming. *Cell Stem Cell*, 17(6), 719-734. <https://doi.org/10.1016/j.stem.2015.09.002>
- Contractor, A., Ethell, I. M., & Portera-Cailliau, C. (2021). Cortical interneurons in autism. *Nat Neurosci*, 24(12), 1648-1659. <https://doi.org/10.1038/s41593-021-00967-6>
- Davis, R. L., Weintraub, H., & Lassar, A. B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell*, 51(6), 987-1000. [https://doi.org/10.1016/0092-8674\(87\)90585-x](https://doi.org/10.1016/0092-8674(87)90585-x)

- Dehorter, N., Ciceri, G., Bartolini, G., Lim, L., del Pino, I., & Marin, O. (2015). Tuning of fast-spiking interneuron properties by an activity-dependent transcriptional switch. *Science*, 349(6253), 1216-1220. <https://doi.org/10.1126/science.aab3415>
- Dimou, L., & Gotz, M. (2014). Glial cells as progenitors and stem cells: new roles in the healthy and diseased brain. *Physiol Rev*, 94(3), 709-737. <https://doi.org/10.1152/physrev.00036.2013>
- Douglas, R. J., Koch, C., Mahowald, M., Martin, K. A., & Suarez, H. H. (1995). Recurrent excitation in neocortical circuits. *Science*, 269(5226), 981-985. <https://doi.org/10.1126/science.7638624>
- Drexel, M., Rahimi, S., & Sperk, G. (2022). Silencing of Hippocampal Somatostatin Interneurons Induces Recurrent Spontaneous Limbic Seizures in Mice. *Neuroscience*, 487, 155-165. <https://doi.org/10.1016/j.neuroscience.2022.02.007>
- Drexel, M., Romanov, R. A., Wood, J., Weger, S., Heilbronn, R., Wulff, P., Tasan, R. O., Harkany, T., & Sperk, G. (2017). Selective Silencing of Hippocampal Parvalbumin Interneurons Induces Development of Recurrent Spontaneous Limbic Seizures in Mice. *J Neurosci*, 37(34), 8166-8179. <https://doi.org/10.1523/JNEUROSCI.3456-16.2017>
- Du, T., Xu, Q., Ocbina, P. J., & Anderson, S. A. (2008). NKX2.1 specifies cortical interneuron fate by activating Lhx6. *Development*, 135(8), 1559-1567. <https://doi.org/10.1242/dev.015123>
- Dubonyte, U., Asenjo-Martinez, A., Werge, T., Lage, K., & Kirkeby, A. (2022). Current advancements of modelling schizophrenia using patient-derived induced pluripotent stem cells. *Acta Neuropathol Commun*, 10(1), 183. <https://doi.org/10.1186/s40478-022-01460-2>
- Eichmuller, O. L., Corsini, N. S., Vertesy, A., Morassut, I., Scholl, T., Gruber, V. E., Peer, A. M., Chu, J., Novatchkova, M., Hainfellner, J. A., Paredes, M. F., Feucht, M., & Knoblich, J. A. (2022). Amplification of human interneuron progenitors promotes brain tumors and neurological defects. *Science*, 375(6579), eabf5546. <https://doi.org/10.1126/science.abf5546>
- Eisenstat, D. D., Liu, J. K., Mione, M., Zhong, W., Yu, G., Anderson, S. A., Ghattas, I., Puellas, L., & Rubenstein, J. L. (1999). DLX-1, DLX-2, and DLX-5 expression define distinct stages of basal forebrain differentiation. *J Comp Neurol*, 414(2), 217-237. [https://doi.org/10.1002/\(sici\)1096-9861\(19991115\)414:2<217::aid-cne6>3.0.co;2-i](https://doi.org/10.1002/(sici)1096-9861(19991115)414:2<217::aid-cne6>3.0.co;2-i)
- Favuzzi, E., Deogracias, R., Marques-Smith, A., Maeso, P., Jezequel, J., Exposito-Alonso, D., Balia, M., Kroon, T., Hinojosa, A. J., E, F. M., & Rico, B. (2019). Distinct molecular programs regulate synapse specificity in cortical inhibitory circuits. *Science*, 363(6425), 413-417. <https://doi.org/10.1126/science.aau8977>
- Fawcett, J. W., Oohashi, T., & Pizzorusso, T. (2019). The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nat Rev Neurosci*, 20(8), 451-465. <https://doi.org/10.1038/s41583-019-0196-3>
- Filice, F., Vorckel, K. J., Sungur, A. O., Wöhr, M., & Schwaller, B. (2016). Reduction in parvalbumin expression not loss of the parvalbumin-expressing GABA interneuron subpopulation in genetic parvalbumin and shank mouse models of autism. *Mol Brain*, 9, 10. <https://doi.org/10.1186/s13041-016-0192-8>

- Fisher, J., Verhagen, M., Long, Z., Moissidis, M., Yan, Y., He, C., Wang, J., Micoli, E., Alastruey, C. M., Moors, R., Marin, O., Mi, D., & Lim, L. (2024). Cortical somatostatin long-range projection neurons and interneurons exhibit divergent developmental trajectories. *Neuron*, 112(4), 558-573 e558. <https://doi.org/10.1016/j.neuron.2023.11.013>
- Flaherty, E., Deranieh, R. M., Artimovich, E., Lee, I. S., Siegel, A. J., Levy, D. L., Nestor, M. W., & Brennand, K. J. (2017). Patient-derived hiPSC neurons with heterozygous CNTNAP2 deletions display altered neuronal gene expression and network activity. *NPJ Schizophr*, 3(1), 35. <https://doi.org/10.1038/s41537-017-0033-5>
- Flames, N., Long, J. E., Garratt, A. N., Fischer, T. M., Gassmann, M., Birchmeier, C., Lai, C., Rubenstein, J. L., & Marin, O. (2004). Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. *Neuron*, 44(2), 251-261. <https://doi.org/10.1016/j.neuron.2004.09.028>
- Flames, N., Pla, R., Gelman, D. M., Rubenstein, J. L., Puelles, L., & Marin, O. (2007). Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. *J Neurosci*, 27(36), 9682-9695. <https://doi.org/10.1523/JNEUROSCI.2750-07.2007>
- Giacomoni, J., Bruzelius, A., Habekost, M., Kajtez, J., Ottosson, D. R., Fiorenzano, A., Storm, P., & Parmar, M. (2024). 3D model for human glia conversion into subtype-specific neurons, including dopamine neurons. *Cell Rep Methods*, 4(9), 100845. <https://doi.org/10.1016/j.crmeth.2024.100845>
- Giacomoni, J., Bruzelius, A., Stamouli, C. A., & Rylander Ottosson, D. (2020). Direct Conversion of Human Stem Cell-Derived Glial Progenitor Cells into GABAergic Interneurons. *Cells*, 9(11). <https://doi.org/10.3390/cells9112451>
- Giacomoni, J., Habekost, M., Cepeda-Prado, E., Mattsson, B., Ottosson, D. R., Parmar, M., & Kajtez, J. (2023). Protocol for optical clearing and imaging of fluorescently labeled ex vivo rat brain slices. *STAR Protoc*, 4(1), 102041. <https://doi.org/10.1016/j.xpro.2022.102041>
- Godoy, L. D., Prizon, T., Rossignoli, M. T., Leite, J. P., & Liberato, J. L. (2022). Parvalbumin Role in Epilepsy and Psychiatric Comorbidities: From Mechanism to Intervention. *Front Integr Neurosci*, 16, 765324. <https://doi.org/10.3389/fnint.2022.765324>
- Guo, Z., Zhang, L., Wu, Z., Chen, Y., Wang, F., & Chen, G. (2014). In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. *Cell Stem Cell*, 14(2), 188-202. <https://doi.org/10.1016/j.stem.2013.12.001>
- Hanson, E., Armbruster, M., Lau, L. A., Sommer, M. E., Klaft, Z. J., Swanger, S. A., Traynelis, S. F., Moss, S. J., Noubary, F., Chadchankar, J., & Dulla, C. G. (2019). Tonic Activation of GluN2C/GluN2D-Containing NMDA Receptors by Ambient Glutamate Facilitates Cortical Interneuron Maturation. *J Neurosci*, 39(19), 3611-3626. <https://doi.org/10.1523/JNEUROSCI.1392-18.2019>

- Hathy, E., Szabo, E., Varga, N., Erdei, Z., Tordai, C., Czehlar, B., Baradits, M., Jezso, B., Koller, J., Nagy, L., Molnar, M. J., Homolya, L., Nemoda, Z., Apati, A., & Rethelyi, J. M. (2020). Investigation of de novo mutations in a schizophrenia case-parent trio by induced pluripotent stem cell-based in vitro disease modeling: convergence of schizophrenia- and autism-related cellular phenotypes. *Stem Cell Res Ther*, 11(1), 504. <https://doi.org/10.1186/s13287-020-01980-5>
- Hathy, E., Szabo, E., Vincze, K., Haltrich, I., Kiss, E., Varga, N., Erdei, Z., Varady, G., Homolya, L., Apati, A., & Rethelyi, J. M. (2021). Generation of multiple iPSC clones from a male schizophrenia patient carrying de novo mutations in genes KHSRP, LRRC7, and KIR2DL1, and his parents. *Stem Cell Res*, 51, 102140. <https://doi.org/10.1016/j.scr.2020.102140>
- He, M., Tucciarone, J., Lee, S., Nigro, M. J., Kim, Y., Levine, J. M., Kelly, S. M., Krugikov, I., Wu, P., Chen, Y., Gong, L., Hou, Y., Osten, P., Rudy, B., & Huang, Z. J. (2016). Strategies and Tools for Combinatorial Targeting of GABAergic Neurons in Mouse Cerebral Cortex. *Neuron*, 91(6), 1228-1243. <https://doi.org/10.1016/j.neuron.2016.08.021>
- Heinrich, C., Bergami, M., Gascon, S., Lepier, A., Vigano, F., Dimou, L., Sutor, B., Berninger, B., & Gotz, M. (2014). Sox2-mediated conversion of NG2 glia into induced neurons in the injured adult cerebral cortex. *Stem Cell Reports*, 3(6), 1000-1014. <https://doi.org/10.1016/j.stemcr.2014.10.007>
- Heinrich, C., Blum, R., Gascon, S., Masserdotti, G., Tripathi, P., Sanchez, R., Tiedt, S., Schroeder, T., Gotz, M., & Berninger, B. (2010). Directing astroglia from the cerebral cortex into subtype specific functional neurons. *PLoS Biol*, 8(5), e1000373. <https://doi.org/10.1371/journal.pbio.1000373>
- Heins, N., Malatesta, P., Cecconi, F., Nakafuku, M., Tucker, K. L., Hack, M. A., Chapouton, P., Barde, Y. A., & Gotz, M. (2002). Glial cells generate neurons: the role of the transcription factor Pax6. *Nat Neurosci*, 5(4), 308-315. <https://doi.org/10.1038/nn828>
- Hendriks, D., Clevers, H., & Artigiani, B. (2020). CRISPR-Cas Tools and Their Application in Genetic Engineering of Human Stem Cells and Organoids. *Cell Stem Cell*, 27(5), 705-731. <https://doi.org/10.1016/j.stem.2020.10.014>
- Herrero-Navarro, A., Puche-Aroca, L., Moreno-Juan, V., Sempere-Ferrandez, A., Espinosa, A., Susin, R., Torres-Masjoan, L., Leyva-Diaz, E., Karow, M., Figueres-Onate, M., Lopez-Mascaraque, L., Lopez-Atalaya, J. P., Berninger, B., & Lopez-Bendito, G. (2021). Astrocytes and neurons share region-specific transcriptional signatures that confer regional identity to neuronal reprogramming. *Sci Adv*, 7(15). <https://doi.org/10.1126/sciadv.abe8978>
- Hodge, R. D., Bakken, T. E., Miller, J. A., Smith, K. A., Barkan, E. R., Graybuck, L. T., Close, J. L., Long, B., Johansen, N., Penn, O., Yao, Z., Eggermont, J., Holtt, T., Levi, B. P., Shehata, S. I., Aevermann, B., Beller, A., Bertagnolli, D., Brouner, K.,...Lein, E. S. (2019). Conserved cell types with divergent features in human versus mouse cortex. *Nature*, 573(7772), 61-68. <https://doi.org/10.1038/s41586-019-1506-7>
- Hu, H., Gan, J., & Jonas, P. (2014). Interneurons. Fast-spiking, parvalbumin(+) GABAergic interneurons: from cellular design to microcircuit function. *Science*, 345(6196), 1255263. <https://doi.org/10.1126/science.1255263>

- Hu, X., Qin, S., Huang, X., Yuan, Y., Tan, Z., Gu, Y., Cheng, X., Wang, D., Lian, X. F., He, C., & Su, Z. (2019). Region-Restrict Astrocytes Exhibit Heterogeneous Susceptibility to Neuronal Reprogramming. *Stem Cell Reports*, 12(2), 290-304. <https://doi.org/10.1016/j.stemcr.2018.12.017>
- Hughes, E. G., Kang, S. H., Fukaya, M., & Bergles, D. E. (2013). Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nat Neurosci*, 16(6), 668-676. <https://doi.org/10.1038/nn.3390>
- Hussman, J. P. (2001). Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. *J Autism Dev Disord*, 31(2), 247-248. <https://doi.org/10.1023/a:1010715619091>
- Inda, M. C., DeFelipe, J., & Munoz, A. (2009). Morphology and distribution of chandelier cell axon terminals in the mouse cerebral cortex and claustroramygdaloid complex. *Cereb Cortex*, 19(1), 41-54. <https://doi.org/10.1093/cercor/bhn057>
- Iwafuchi-Doi, M., & Zaret, K. S. (2014). Pioneer transcription factors in cell reprogramming. *Genes Dev*, 28(24), 2679-2692. <https://doi.org/10.1101/gad.253443.114>
- Joshi, D., Catts, V. S., Olaya, J. C., & Shannon Weickert, C. (2015). Relationship between somatostatin and death receptor expression in the orbital frontal cortex in schizophrenia: a postmortem brain mRNA study. *NPJ Schizophr*, 1, 14004. <https://doi.org/10.1038/npschz.2014.4>
- Kajtez, J., Laurin, K., Nilsson, F., Bruzelius, A., Cepeda-Prado, E., Birtele, M., Barker, R. A., Herborg, F., Rylander Ottosson, D., Storm, P., Fiorenzano, A., Habekost, M., & Parmar, M. (2025). Three-dimensional cell-cell interactions promote direct reprogramming of patient fibroblasts into functional and transplantable neurons. *Sci Adv*, 11(23), eadq7855. <https://doi.org/10.1126/sciadv.adq7855>
- Karow, M., Camp, J. G., Falk, S., Gerber, T., Pataskar, A., Gac-Santel, M., Kageyama, J., Brazovskaja, A., Garding, A., Fan, W., Riedemann, T., Casamassa, A., Smiyakin, A., Schichor, C., Gotz, M., Tiwari, V. K., Treutlein, B., & Berninger, B. (2018). Direct pericyte-to-neuron reprogramming via unfolding of a neural stem cell-like program. *Nat Neurosci*, 21(7), 932-940. <https://doi.org/10.1038/s41593-018-0168-3>
- Kathuria, A., Lopez-Lengowski, K., Watmuff, B., McPhie, D., Cohen, B. M., & Karmacharya, R. (2019). Synaptic deficits in iPSC-derived cortical interneurons in schizophrenia are mediated by NLGN2 and rescued by N-acetylcysteine. *Transl Psychiatry*, 9(1), 321. <https://doi.org/10.1038/s41398-019-0660-x>
- Kepecs, A., & Fishell, G. (2014). Interneuron cell types are fit to function. *Nature*, 505(7483), 318-326. <https://doi.org/10.1038/nature12983>
- Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M. J., Ji, H., Ehrlich, L. I., Yabuuchi, A., Takeuchi, A., Cuniff, K. C., Hongguang, H., McKinney-Freeman, S., Naveiras, O., Yoon, T. J., Irizarry, R. A., Jung, N.,... Daley, G. Q. (2010). Epigenetic memory in induced pluripotent stem cells. *Nature*, 467(7313), 285-290. <https://doi.org/10.1038/nature09342>
- Kim, T. G., Yao, R., Monnell, T., Cho, J. H., Vasudevan, A., Koh, A., Peeyush, K. T., Moon, M., Datta, D., Bolshakov, V. Y., Kim, K. S., & Chung, S. (2014). Efficient specification of interneurons from human pluripotent stem cells by dorsoventral and rostrocaudal modulation. *Stem Cells*, 32(7), 1789-1804. <https://doi.org/10.1002/stem.1704>

- Kistemaker, L., van Bodegraven, E. J., de Vries, H. E., & Hol, E. M. (2025). Vascularized human brain organoids: current possibilities and prospects. *Trends Biotechnol*, 43(6), 1275-1285. <https://doi.org/10.1016/j.tibtech.2024.11.021>
- Krienen, F. M., Goldman, M., Zhang, Q., R, C. H. D. R., Florio, M., Machold, R., Saunders, A., Levandowski, K., Zaniewski, H., Schuman, B., Wu, C., Lutservitz, A., Mullally, C. D., Reed, N., Bien, E., Bortolin, L., Fernandez-Otero, M., Lin, J. D., Wysoker, A.,...McCarroll, S. A. (2020). Innovations present in the primate interneuron repertoire. *Nature*, 586(7828), 262-269. <https://doi.org/10.1038/s41586-020-2781-z>
- Leitch, B. (2024). Parvalbumin Interneuron Dysfunction in Neurological Disorders: Focus on Epilepsy and Alzheimer's Disease. *Int J Mol Sci*, 25(10). <https://doi.org/10.3390/ijms25105549>
- Lentini, C., d'Orange, M., Marichal, N., Trottmann, M. M., Vignoles, R., Foucault, L., Verrier, C., Massera, C., Raineteau, O., Conzelmann, K. K., Rival-Gervier, S., Depaulis, A., Berninger, B., & Heinrich, C. (2021). Reprogramming reactive glia into interneurons reduces chronic seizure activity in a mouse model of mesial temporal lobe epilepsy. *Cell Stem Cell*, 28(12), 2104-2121 e2110. <https://doi.org/10.1016/j.stem.2021.09.002>
- Levy, R. J., & Pasca, S. P. (2025). From Organoids to Assembloids: Experimental Approaches to Study Human Neuropsychiatric Disorders. *Annu Rev Neurosci*, 48(1), 363-379. <https://doi.org/10.1146/annurev-neuro-112723-023232>
- Lewis, D. A., Hashimoto, T., & Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*, 6(4), 312-324. <https://doi.org/10.1038/nrn1648>
- Li, D., Wu, Q., & Han, X. (2022). Application of Medial Ganglionic Eminence Cell Transplantation in Diseases Associated With Interneuron Disorders. *Front Cell Neurosci*, 16, 939294. <https://doi.org/10.3389/fncel.2022.939294>
- Lim, L., Mi, D., Llorca, A., & Marin, O. (2018). Development and Functional Diversification of Cortical Interneurons. *Neuron*, 100(2), 294-313. <https://doi.org/10.1016/j.neuron.2018.10.009>
- Lin, M., Pedrosa, E., Hrabovsky, A., Chen, J., Puliafito, B. R., Gilbert, S. R., Zheng, D., & Lachman, H. M. (2016). Integrative transcriptome network analysis of iPSC-derived neurons from schizophrenia and schizoaffective disorder patients with 22q11.2 deletion. *BMC Syst Biol*, 10(1), 105. <https://doi.org/10.1186/s12918-016-0366-0>
- Liodis, P., Denaxa, M., Grigoriou, M., Akufo-Addo, C., Yanagawa, Y., & Pachnis, V. (2007). Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. *J Neurosci*, 27(12), 3078-3089. <https://doi.org/10.1523/JNEUROSCI.3055-06.2007>
- Liu, M. L., Zang, T., Zou, Y., Chang, J. C., Gibson, J. R., Huber, K. M., & Zhang, C. L. (2013). Small molecules enable neurogenin 2 to efficiently convert human fibroblasts into cholinergic neurons. *Nat Commun*, 4, 2183. <https://doi.org/10.1038/ncomms3183>
- Liu, W. S., Pesold, C., Rodriguez, M. A., Carboni, G., Auta, J., Lacor, P., Larson, J., Condie, B. G., Guidotti, A., & Costa, E. (2001). Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. *Proc Natl Acad Sci U S A*, 98(6), 3477-3482. <https://doi.org/10.1073/pnas.051614698>

- Liu, Y., Liu, H., Sauvey, C., Yao, L., Zarnowska, E. D., & Zhang, S. C. (2013). Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. *Nat Protoc*, 8(9), 1670-1679. <https://doi.org/10.1038/nprot.2013.106>
- Llorca, A., & Deogracias, R. (2022). Origin, Development, and Synaptogenesis of Cortical Interneurons. *Front Neurosci*, 16, 929469. <https://doi.org/10.3389/fnins.2022.929469>
- Mariani, J., Coppola, G., Zhang, P., Abyzov, A., Provini, L., Tomasini, L., Amenduni, M., Szekely, A., Palejev, D., Wilson, M., Gerstein, M., Grigorenko, E. L., Chawarska, K., Pelphrey, K. A., Howe, J. R., & Vaccarino, F. M. (2015). FOXP1-Dependent Dysregulation of GABA/Glutamate Neuron Differentiation in Autism Spectrum Disorders. *Cell*, 162(2), 375-390. <https://doi.org/10.1016/j.cell.2015.06.034>
- Marichal, N., Peron, S., Beltran Arranz, A., Galante, C., Franco Scarante, F., Wiffen, R., Schuurmans, C., Karow, M., Gascon, S., & Berninger, B. (2024). Reprogramming astroglia into neurons with hallmarks of fast-spiking parvalbumin-positive interneurons by phospho-site-deficient Ascl1. *Sci Adv*, 10(43), ead15935. <https://doi.org/10.1126/sciadv.adl5935>
- Marin, O. (2012). Interneuron dysfunction in psychiatric disorders. *Nat Rev Neurosci*, 13(2), 107-120. <https://doi.org/10.1038/nrn3155>
- Marin, O. (2024). Parvalbumin interneuron deficits in schizophrenia. *Eur Neuropsychopharmacol*, 82, 44-52. <https://doi.org/10.1016/j.euroneuro.2024.02.010>
- Marin, O. (2025). Development of GABAergic Interneurons in the Human Cerebral Cortex. *Eur J Neurosci*, 61(9), e70136. <https://doi.org/10.1111/ejn.70136>
- Marin, O., Yaron, A., Bagri, A., Tessier-Lavigne, M., & Rubenstein, J. L. (2001). Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. *Science*, 293(5531), 872-875. <https://doi.org/10.1126/science.1061891>
- Maroof, A. M., Keros, S., Tyson, J. A., Ying, S. W., Ganat, Y. M., Merkle, F. T., Liu, B., Goulburn, A., Stanley, E. G., Elefanty, A. G., Widmer, H. R., Eggan, K., Goldstein, P. A., Anderson, S. A., & Studer, L. (2013). Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. *Cell Stem Cell*, 12(5), 559-572. <https://doi.org/10.1016/j.stem.2013.04.008>
- Marques-Smith, A., Lyngholm, D., Kaufmann, A. K., Stacey, J. A., Hoerder-Suabedissen, A., Becker, E. B., Wilson, M. C., Molnar, Z., & Butt, S. J. (2016). A Transient Translaminar GABAergic Interneuron Circuit Connects Thalamocortical Recipient Layers in Neonatal Somatosensory Cortex. *Neuron*, 89(3), 536-549. <https://doi.org/10.1016/j.neuron.2016.01.015>
- Masserdotti, G., Gillotin, S., Sutor, B., Drechsel, D., Irmeler, M., Jorgensen, H. F., Sass, S., Theis, F. J., Beckers, J., Berninger, B., Guillemot, F., & Gotz, M. (2015). Transcriptional Mechanisms of Proneural Factors and REST in Regulating Neuronal Reprogramming of Astrocytes. *Cell Stem Cell*, 17(1), 74-88. <https://doi.org/10.1016/j.stem.2015.05.014>
- Matsuda, T., Irie, T., Katsurabayashi, S., Hayashi, Y., Nagai, T., Hamazaki, N., Adefuin, A. M. D., Miura, F., Ito, T., Kimura, H., Shirahige, K., Takeda, T., Iwasaki, K., Imamura, T., & Nakashima, K. (2019). Pioneer Factor NeuroD1 Rearranges Transcriptional and Epigenetic Profiles to Execute Microglia-Neuron Conversion. *Neuron*, 101(3), 472-485 e477. <https://doi.org/10.1016/j.neuron.2018.12.010>

- Mattugini, N., Bocchi, R., Scheuss, V., Russo, G. L., Torper, O., Lao, C. L., & Gotz, M. (2019). Inducing Different Neuronal Subtypes from Astrocytes in the Injured Mouse Cerebral Cortex. *Neuron*, 103(6), 1086-1095 e1085. <https://doi.org/10.1016/j.neuron.2019.08.009>
- Miyoshi, G., Butt, S. J., Takebayashi, H., & Fishell, G. (2007). Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. *J Neurosci*, 27(29), 7786-7798. <https://doi.org/10.1523/JNEUROSCI.1807-07.2007>
- Moissidis, M., Abbasova, L., Selten, M., Alis, R., Bernard, C., Dominguez-Canterla, Y., Oozer, F., Qin, S., Kelly, A., Modol, L., Vasistha, N. A., Jones, B., Dhami, P., Khodosevich, K., Hamid, F., Lavender, P., Flames, N., & Marin, O. (2025). A postnatal molecular switch drives activity-dependent maturation of parvalbumin interneurons. *Cell*, 188(20), 5555-5575 e5526. <https://doi.org/10.1016/j.cell.2025.06.029>
- Moriarty, N., Fraser, T. D., Hunt, C. P. J., Eleftheriou, G., Kauhausen, J. A., Thompson, L. H., & Parish, C. L. (2025). Exercise promotes the functional integration of human stem cell-derived neural grafts in a rodent model of Parkinson's disease. *Stem Cell Reports*, 20(5), 102480. <https://doi.org/10.1016/j.stemcr.2025.102480>
- Mostajo-Radji, M. A., Leon, W. R. M., Breevoort, A., Gonzalez-Ferrer, J., Schweiger, H. E., Lehrer, J., Zhou, L., Schmitz, M. T., Perez, Y., Mukhtar, T., Robbins, A., Chu, J., Andrews, M. G., Sullivan, F. N., Tejera, D., Choy, E. C., Paredes, M. F., Teodorescu, M., Kriegstein, A. R.,...Pollen, A. A. (2025). Fate plasticity of interneuron specification. *iScience*, 28(4), 112295. <https://doi.org/10.1016/j.isci.2025.112295>
- Ni, P., Noh, H., Park, G. H., Shao, Z., Guan, Y., Park, J. M., Yu, S., Park, J. S., Coyle, J. T., Weinberger, D. R., Straub, R. E., Cohen, B. M., McPhie, D. L., Yin, C., Huang, W., Kim, H. Y., & Chung, S. (2020). iPSC-derived homogeneous populations of developing schizophrenia cortical interneurons have compromised mitochondrial function. *Mol Psychiatry*, 25(11), 2873-2888. <https://doi.org/10.1038/s41380-019-0423-3>
- Ni, P., Noh, H., Shao, Z., Zhu, Q., Guan, Y., Park, J. J., Arif, F., Park, J. M., Abani, C., Beaudreault, C., Park, J. S., Berry, E., Moghadam, A., Stanton, P., Hutchinson, J. N., Andrews, B., Faux, C., Parnevelas, J., Eisenberg, L. M.,...Chung, S. (2019). Large-Scale Generation and Characterization of Homogeneous Populations of Migratory Cortical Interneurons from Human Pluripotent Stem Cells. *Mol Ther Methods Clin Dev*, 13, 414-430. <https://doi.org/10.1016/j.omtm.2019.04.002>
- Nicholas, C. R., Chen, J., Tang, Y., Southwell, D. G., Chalmers, N., Vogt, D., Arnold, C. M., Chen, Y. J., Stanley, E. G., Elefanty, A. G., Sasai, Y., Alvarez-Buylla, A., Rubenstein, J. L., & Kriegstein, A. R. (2013). Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell*, 12(5), 573-586. <https://doi.org/10.1016/j.stem.2013.04.005>
- Nigro, M. J., Hashikawa-Yamasaki, Y., & Rudy, B. (2018). Diversity and Connectivity of Layer 5 Somatostatin-Expressing Interneurons in the Mouse Barrel Cortex. *J Neurosci*, 38(7), 1622-1633. <https://doi.org/10.1523/JNEUROSCI.2415-17.2017>
- Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S., & Kriegstein, A. R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature*, 409(6821), 714-720. <https://doi.org/10.1038/35055553>

- Nolbrant, S., Giacomoni, J., Hoban, D. B., Bruzelius, A., Birtele, M., Chandler-Militello, D., Pereira, M., Ottosson, D. R., Goldman, S. A., & Parmar, M. (2020). Direct Reprogramming of Human Fetal- and Stem Cell-Derived Glial Progenitor Cells into Midbrain Dopaminergic Neurons. *Stem Cell Reports*, 15(4), 869-882. <https://doi.org/10.1016/j.stemcr.2020.08.013>
- Okano, H., & Morimoto, S. (2022). iPSC-based disease modeling and drug discovery in cardinal neurodegenerative disorders. *Cell Stem Cell*, 29(2), 189-208. <https://doi.org/10.1016/j.stem.2022.01.007>
- Pagani, M., Bertero, A., Liska, A., Galbusera, A., Sabbioni, M., Barsotti, N., Colenbier, N., Marinazzo, D., Scattoni, M. L., Pasqualetti, M., & Gozzi, A. (2019). Deletion of Autism Risk Gene Shank3 Disrupts Prefrontal Connectivity. *J Neurosci*, 39(27), 5299-5310. <https://doi.org/10.1523/JNEUROSCI.2529-18.2019>
- Pai, E. L., Chen, J., Fazel Darbandi, S., Cho, F. S., Chen, J., Lindtner, S., Chu, J. S., Paz, J. T., Vogt, D., Paredes, M. F., & Rubenstein, J. L. (2020). Maf and Mafb control mouse pallial interneuron fate and maturation through neuropsychiatric disease gene regulation. *Elife*, 9. <https://doi.org/10.7554/eLife.54903>
- Pai, E. L., Vogt, D., Clemente-Perez, A., McKinsey, G. L., Cho, F. S., Hu, J. S., Wimer, M., Paul, A., Fazel Darbandi, S., Pla, R., Nowakowski, T. J., Goodrich, L. V., Paz, J. T., & Rubenstein, J. L. R. (2019). Mafb and c-Maf Have Prenatal Compensatory and Postnatal Antagonistic Roles in Cortical Interneuron Fate and Function. *Cell Rep*, 26(5), 1157-1173 e1155. <https://doi.org/10.1016/j.celrep.2019.01.031>
- Pan-Vazquez, A., Wefelmeyer, W., Gonzalez Sabater, V., Neves, G., & Burrone, J. (2020). Activity-Dependent Plasticity of Axo-axonic Synapses at the Axon Initial Segment. *Neuron*, 106(2), 265-276 e266. <https://doi.org/10.1016/j.neuron.2020.01.037>
- Pang, Z. P., Yang, N., Vierbuchen, T., Ostermeier, A., Fuentes, D. R., Yang, T. Q., Citri, A., Sebastiano, V., Marro, S., Sudhof, T. C., & Wernig, M. (2011). Induction of human neuronal cells by defined transcription factors. *Nature*, 476(7359), 220-223. <https://doi.org/10.1038/nature10202>
- Park, G. H., Noh, H., Shao, Z., Ni, P., Qin, Y., Liu, D., Beaudreault, C. P., Park, J. S., Abani, C. P., Park, J. M., Le, D. T., Gonzalez, S. Z., Guan, Y., Cohen, B. M., McPhie, D. L., Coyle, J. T., Lanz, T. A., Xi, H. S., Yin, C.,...Chung, S. (2020). Activated microglia cause metabolic disruptions in developmental cortical interneurons that persist in interneurons from individuals with schizophrenia. *Nat Neurosci*, 23(11), 1352-1364. <https://doi.org/10.1038/s41593-020-00724-1>
- Penagarikano, O., Abrahams, B. S., Herman, E. I., Winden, K. D., Gdalyahu, A., Dong, H., Sonnenblick, L. I., Gruver, R., Almajano, J., Bragin, A., Golshani, P., Trachtenberg, J. T., Peles, E., & Geschwind, D. H. (2011). Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell*, 147(1), 235-246. <https://doi.org/10.1016/j.cell.2011.08.040>
- Pereira, M., Birtele, M., & Rylander Ottosson, D. (2019). Direct reprogramming into interneurons: potential for brain repair. *Cell Mol Life Sci*, 76(20), 3953-3967. <https://doi.org/10.1007/s00018-019-03193-3>

- Pereira, M., Birtele, M., Shrigley, S., Benitez, J. A., Hedlund, E., Parmar, M., & Ottosson, D. R. (2017). Direct Reprogramming of Resident NG2 Glia into Neurons with Properties of Fast-Spiking Parvalbumin-Containing Interneurons. *Stem Cell Reports*, 9(3), 742-751. <https://doi.org/10.1016/j.stemcr.2017.07.023>
- Pfisterer, U., Kirkeby, A., Torper, O., Wood, J., Nelander, J., Dufour, A., Bjorklund, A., Lindvall, O., Jakobsson, J., & Parmar, M. (2011). Direct conversion of human fibroblasts to dopaminergic neurons. *Proc Natl Acad Sci U S A*, 108(25), 10343-10348. <https://doi.org/10.1073/pnas.1105135108>
- Polleux, F., Whitford, K. L., Dijkhuizen, P. A., Vitalis, T., & Ghosh, A. (2002). Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. *Development*, 129(13), 3147-3160. <https://doi.org/10.1242/dev.129.13.3147>
- Powell, E. M., Mars, W. M., & Levitt, P. (2001). Hepatocyte growth factor/scatter factor is a motogen for interneurons migrating from the ventral to dorsal telencephalon. *Neuron*, 30(1), 79-89. [https://doi.org/10.1016/s0896-6273\(01\)00264-1](https://doi.org/10.1016/s0896-6273(01)00264-1)
- Pozas, E., & Ibanez, C. F. (2005). GDNF and GFRalpha1 promote differentiation and tangential migration of cortical GABAergic neurons. *Neuron*, 45(5), 701-713. <https://doi.org/10.1016/j.neuron.2005.01.043>
- Rivetti di Val Cervo, P., Romanov, R. A., Spigolon, G., Masini, D., Martin-Montanez, E., Toledo, E. M., La Manno, G., Feyder, M., Pifl, C., Ng, Y. H., Sanchez, S. P., Linnarsson, S., Wernig, M., Harkany, T., Fisone, G., & Arenas, E. (2017). Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. *Nat Biotechnol*, 35(5), 444-452. <https://doi.org/10.1038/nbt.3835>
- Robel, S., Berninger, B., & Gotz, M. (2011). The stem cell potential of glia: lessons from reactive gliosis. *Nat Rev Neurosci*, 12(2), 88-104. <https://doi.org/10.1038/nrn2978>
- Roussos, P., Guenewig, B., Kaczorowski, D. C., Barry, G., & Brennand, K. J. (2016). Activity-Dependent Changes in Gene Expression in Schizophrenia Human-Induced Pluripotent Stem Cell Neurons. *JAMA Psychiatry*, 73(11), 1180-1188. <https://doi.org/10.1001/jamapsychiatry.2016.2575>
- Sabogal-Guaqueta, A. M., Mitchell-Garcia, T., Hunneman, J., Voshart, D., Thiruvalluvan, A., Foijer, F., Kruyt, F., Trombetta-Lima, M., Eggen, B. J. L., Boddeke, E., Barazzuol, L., & Dolga, A. M. (2024). Brain organoid models for studying the function of iPSC-derived microglia in neurodegeneration and brain tumours. *Neurobiol Dis*, 203, 106742. <https://doi.org/10.1016/j.nbd.2024.106742>
- Shao, Z., Noh, H., Bin Kim, W., Ni, P., Nguyen, C., Cote, S. E., Noyes, E., Zhao, J., Parsons, T., Park, J. M., Zheng, K., Park, J. J., Coyle, J. T., Weinberger, D. R., Straub, R. E., Berman, K. F., Apud, J., Ongur, D., Cohen, B. M.,... Chung, S. (2019). Dysregulated protocadherin-pathway activity as an intrinsic defect in induced pluripotent stem cell-derived cortical interneurons from subjects with schizophrenia. *Nat Neurosci*, 22(2), 229-242. <https://doi.org/10.1038/s41593-018-0313-z>
- Shi, Z., Zhang, J., Chen, S., Li, Y., Lei, X., Qiao, H., Zhu, Q., Hu, B., Zhou, Q., & Jiao, J. (2016). Conversion of Fibroblasts to Parvalbumin Neurons by One Transcription Factor, Ascl1, and the Chemical Compound Forskolin. *J Biol Chem*, 291(26), 13560-13570. <https://doi.org/10.1074/jbc.M115.709808>

- Sodersten, E., Toskas, K., Rraklli, V., Tiklova, K., Bjorklund, A. K., Ringner, M., Perlmann, T., & Holmberg, J. (2018). A comprehensive map coupling histone modifications with gene regulation in adult dopaminergic and serotonergic neurons. *Nat Commun*, 9(1), 1226. <https://doi.org/10.1038/s41467-018-03538-9>
- Soldner, F., & Jaenisch, R. (2012). Medicine. iPSC disease modeling. *Science*, 338(6111), 1155-1156. <https://doi.org/10.1126/science.1227682>
- Southwell, D. G., Nicholas, C. R., Basbaum, A. I., Stryker, M. P., Kriegstein, A. R., Rubenstein, J. L., & Alvarez-Buylla, A. (2014). Interneurons from embryonic development to cell-based therapy. *Science*, 344(6180), 1240622. <https://doi.org/10.1126/science.1240622>
- Southwell, D. G., Paredes, M. F., Galvao, R. P., Jones, D. L., Froemke, R. C., Sebe, J. Y., Alfaro-Cervello, C., Tang, Y., Garcia-Verdugo, J. M., Rubenstein, J. L., Baraban, S. C., & Alvarez-Buylla, A. (2012). Intrinsically determined cell death of developing cortical interneurons. *Nature*, 491(7422), 109-113. <https://doi.org/10.1038/nature11523>
- Sterlini, B., Fruscione, F., Baldassari, S., Benfenati, F., Zara, F., & Corradi, A. (2020). Progress of Induced Pluripotent Stem Cell Technologies to Understand Genetic Epilepsy. *Int J Mol Sci*, 21(2). <https://doi.org/10.3390/ijms21020482>
- Stuhmer, T., Anderson, S. A., Ekker, M., & Rubenstein, J. L. (2002). Ectopic expression of the *Dlx* genes induces glutamic acid decarboxylase and *Dlx* expression. *Development*, 129(1), 245-252. <https://doi.org/10.1242/dev.129.1.245>
- Sultan, K. T., Brown, K. N., & Shi, S. H. (2013). Production and organization of neocortical interneurons. *Front Cell Neurosci*, 7, 221. <https://doi.org/10.3389/fncel.2013.00221>
- Sussel, L., Marin, O., Kimura, S., & Rubenstein, J. L. (1999). Loss of *Nkx2.1* homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development*, 126(15), 3359-3370. <https://doi.org/10.1242/dev.126.15.3359>
- Tai, C., Abe, Y., Westenbroek, R. E., Scheuer, T., & Catterall, W. A. (2014). Impaired excitability of somatostatin- and parvalbumin-expressing cortical interneurons in a mouse model of Dravet syndrome. *Proc Natl Acad Sci U S A*, 111(30), E3139-3148. <https://doi.org/10.1073/pnas.1411131111>
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131(5), 861-872. <https://doi.org/10.1016/j.cell.2007.11.019>
- Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126(4), 663-676. <https://doi.org/10.1016/j.cell.2006.07.024>
- Takahashi, K., & Yamanaka, S. (2016). A decade of transcription factor-mediated reprogramming to pluripotency. *Nat Rev Mol Cell Biol*, 17(3), 183-193. <https://doi.org/10.1038/nrm.2016.8>
- Takebe, T., & Wells, J. M. (2019). Organoids by design. *Science*, 364(6444), 956-959. <https://doi.org/10.1126/science.aaw7567>
- Taniguchi, H., Lu, J., & Huang, Z. J. (2013). The spatial and temporal origin of chandelier cells in mouse neocortex. *Science*, 339(6115), 70-74. <https://doi.org/10.1126/science.1227622>

- Tasic, B., Menon, V., Nguyen, T. N., Kim, T. K., Jarsky, T., Yao, Z., Levi, B., Gray, L. T., Sorensen, S. A., Dolbeare, T., Bertagnolli, D., Goldy, J., Shapovalova, N., Parry, S., Lee, C., Smith, K., Bernard, A., Madisen, L., Sunkin, S. M.,...Zeng, H. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nat Neurosci*, 19(2), 335-346. <https://doi.org/10.1038/nn.4216>
- Tasic, B., Yao, Z., Graybuck, L. T., Smith, K. A., Nguyen, T. N., Bertagnolli, D., Goldy, J., Garren, E., Economo, M. N., Viswanathan, S., Penn, O., Bakken, T., Menon, V., Miller, J., Fong, O., Hirokawa, K. E., Lathia, K., Rimorin, C., Tieu, M.,...Zeng, H. (2018). Shared and distinct transcriptomic cell types across neocortical areas. *Nature*, 563(7729), 72-78. <https://doi.org/10.1038/s41586-018-0654-5>
- Torper, O., Ottosson, D. R., Pereira, M., Lau, S., Cardoso, T., Grealish, S., & Parmar, M. (2015). In Vivo Reprogramming of Striatal NG2 Glia into Functional Neurons that Integrate into Local Host Circuitry. *Cell Rep*, 12(3), 474-481. <https://doi.org/10.1016/j.celrep.2015.06.040>
- Torper, O., Pfisterer, U., Wolf, D. A., Pereira, M., Lau, S., Jakobsson, J., Bjorklund, A., Grealish, S., & Parmar, M. (2013). Generation of induced neurons via direct conversion in vivo. *Proc Natl Acad Sci U S A*, 110(17), 7038-7043. <https://doi.org/10.1073/pnas.1303829110>
- Treiman, D. M. (2001). GABAergic mechanisms in epilepsy. *Epilepsia*, 42 Suppl 3, 8-12. <https://doi.org/10.1046/j.1528-1157.2001.042suppl.3008.x>
- Tremblay, R., Lee, S., & Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron*, 91(2), 260-292. <https://doi.org/10.1016/j.neuron.2016.06.033>
- Treutlein, B., Lee, Q. Y., Camp, J. G., Mall, M., Koh, W., Shariati, S. A., Sim, S., Neff, N. F., Skotheim, J. M., Wernig, M., & Quake, S. R. (2016). Dissecting direct reprogramming from fibroblast to neuron using single-cell RNA-seq. *Nature*, 534(7607), 391-395. <https://doi.org/10.1038/nature18323>
- Varela, M. C., Walker, M. P., Bok, J., Crespo, E. L., Thenstedt, T., Goldstein, L., Tidball, A. M., Yuan, Y., Isom, L. L., Fu, J., Uhler, M., & Parent, J. M. (2025). Human Medial Ganglionic Eminence Organoids Robustly Generate Parvalbumin Interneurons and Fast-Spiking Neurons and Reveal Migratory Deficits in SLC6A1 Deficient Interneurons. *bioRxiv*. <https://doi.org/10.1101/2025.07.01.662594>
- Velmeshev, D., Perez, Y., Yan, Z., Valencia, J. E., Castaneda-Castellanos, D. R., Wang, L., Schirmer, L., Mayer, S., Wick, B., Wang, S., Nowakowski, T. J., Paredes, M., Huang, E. J., & Kriegstein, A. R. (2023). Single-cell analysis of prenatal and postnatal human cortical development. *Science*, 382(6667), eadf0834. <https://doi.org/10.1126/science.adf0834>
- Victor, M. B., Richner, M., Hermansteyne, T. O., Ransdell, J. L., Sobieski, C., Deng, P. Y., Klyachko, V. A., Nerbonne, J. M., & Yoo, A. S. (2014). Generation of human striatal neurons by microRNA-dependent direct conversion of fibroblasts. *Neuron*, 84(2), 311-323. <https://doi.org/10.1016/j.neuron.2014.10.016>
- Vid Prkacin, M., Banovac, I., Petanjek, Z., & Hladnik, A. (2023). Cortical interneurons in schizophrenia - cause or effect? *Croat Med J*, 64(2), 110-122. <https://doi.org/10.3325/cmj.2023.64.110>

- Vierbuchen, T., Ostermeier, A., Pang, Z. P., Kokubu, Y., Sudhof, T. C., & Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*, 463(7284), 1035-1041. <https://doi.org/10.1038/nature08797>
- Vignoles, R., Lentini, C., d'Orange, M., & Heinrich, C. (2019). Direct Lineage Reprogramming for Brain Repair: Breakthroughs and Challenges. *Trends Mol Med*, 25(10), 897-914. <https://doi.org/10.1016/j.molmed.2019.06.006>
- Vogt, D., Cho, K. K. A., Shelton, S. M., Paul, A., Huang, Z. J., Sohal, V. S., & Rubenstein, J. L. R. (2018). Mouse Cntnap2 and Human CNTNAP2 ASD Alleles Cell Autonomously Regulate PV+ Cortical Interneurons. *Cereb Cortex*, 28(11), 3868-3879. <https://doi.org/10.1093/cercor/bhx248>
- Vogt, D., Hunt, R. F., Mandal, S., Sandberg, M., Silberberg, S. N., Nagasawa, T., Yang, Z., Baraban, S. C., & Rubenstein, J. L. (2014). Lhx6 directly regulates Arx and CXCR7 to determine cortical interneuron fate and laminar position. *Neuron*, 82(2), 350-364. <https://doi.org/10.1016/j.neuron.2014.02.030>
- Waddington, C. H. (1957). A discussion of some aspects of theoretical biology. *The strategy of the genes*, 905-1975.
- Walsh, R. M., Crabtree, G. W., Kalpana, K., Jubierre, L., Koo, S. Y., Ciceri, G., Gogos, J. A., Kruglikov, I., & Studer, L. (2025). Forebrain assembloids support the development of fast-spiking human PVALB+ cortical interneurons and uncover schizophrenia-associated defects. *Neuron*, 113(19), 3185-3203 e3187. <https://doi.org/10.1016/j.neuron.2025.06.017>
- Wang, H., Yang, Y., Liu, J., & Qian, L. (2021). Direct cell reprogramming: approaches, mechanisms and progress. *Nat Rev Mol Cell Biol*, 22(6), 410-424. <https://doi.org/10.1038/s41580-021-00335-z>
- Wang, L., Yao, Y., Xie, B., Lei, M., Li, Y., Shi, J., Yu, L., Zhou, W., Sang, Y., Kong, L., Liu, H., & Qiu, J. (2025). Nanoelectrode-Mediated Extracellular Electrical Stimulation Directing Dopaminergic Neuronal Differentiation of Stem Cells for Improved Parkinson's Disease Therapy. *Adv Mater*, 37(6), e2409745. <https://doi.org/10.1002/adma.202409745>
- Wang, M., Wei, P. C., Lim, C. K., Gallina, I. S., Marshall, S., Marchetto, M. C., Alt, F. W., & Gage, F. H. (2020). Increased Neural Progenitor Proliferation in a hiPSC Model of Autism Induces Replication Stress-Associated Genome Instability. *Cell Stem Cell*, 26(2), 221-233 e226. <https://doi.org/10.1016/j.stem.2019.12.013>
- Wang, P., Mokhtari, R., Pedrosa, E., Kirschenbaum, M., Bayrak, C., Zheng, D., & Lachman, H. M. (2017). CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells. *Mol Autism*, 8, 11. <https://doi.org/10.1186/s13229-017-0124-1>
- Wang, S., Bates, J., Li, X., Schanz, S., Chandler-Militello, D., Levine, C., Maherali, N., Studer, L., Hochedlinger, K., Windrem, M., & Goldman, S. A. (2013). Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell*, 12(2), 252-264. <https://doi.org/10.1016/j.stem.2012.12.002>

- Wang, X. J., Tegner, J., Constantinidis, C., & Goldman-Rakic, P. S. (2004). Division of labor among distinct subtypes of inhibitory neurons in a cortical microcircuit of working memory. *Proc Natl Acad Sci U S A*, *101*(5), 1368-1373. <https://doi.org/10.1073/pnas.0305337101>
- Wang, Y., Toledo-Rodriguez, M., Gupta, A., Wu, C., Silberberg, G., Luo, J., & Markram, H. (2004). Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. *J Physiol*, *561*(Pt 1), 65-90. <https://doi.org/10.1113/jphysiol.2004.073353>
- Wang, Z., Zheng, D., Tan, Y. S., Yuan, Q., Yuan, F., & Zhang, S. C. (2023). Enabling Survival of Transplanted Neural Precursor Cells in the Ischemic Brain. *Adv Sci (Weinh)*, *10*(33), e2302527. <https://doi.org/10.1002/advs.202302527>
- Wapinski, O. L., Vierbuchen, T., Qu, K., Lee, Q. Y., Chanda, S., Fuentes, D. R., Giresi, P. G., Ng, Y. H., Marro, S., Neff, N. F., Drechsel, D., Martynoga, B., Castro, D. S., Webb, A. E., Sudhof, T. C., Brunet, A., Guillemot, F., Chang, H. Y., & Wernig, M. (2013). Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. *Cell*, *155*(3), 621-635. <https://doi.org/10.1016/j.cell.2013.09.028>
- Wichterle, H., Alvarez-Dolado, M., Erskine, L., & Alvarez-Buylla, A. (2003). Permissive corridor and diffusible gradients direct medial ganglionic eminence cell migration to the neocortex. *Proc Natl Acad Sci U S A*, *100*(2), 727-732. <https://doi.org/10.1073/pnas.242721899>
- Winkel, F., Ryazantseva, M., Voigt, M. B., Didio, G., Lilja, A., Llach Pou, M., Steinzeig, A., Harkki, J., Englund, J., Khirug, S., Rivera, C., Palva, S., Taira, T., Lauri, S. E., Umemori, J., & Castren, E. (2021). Pharmacological and optical activation of TrkB in Parvalbumin interneurons regulate intrinsic states to orchestrate cortical plasticity. *Mol Psychiatry*, *26*(12), 7247-7256. <https://doi.org/10.1038/s41380-021-01211-0>
- Wonders, C. P., Taylor, L., Welagen, J., Mbata, I. C., Xiang, J. Z., & Anderson, S. A. (2008). A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. *Dev Biol*, *314*(1), 127-136. <https://doi.org/10.1016/j.ydbio.2007.11.018>
- Wong, F. K., Bercsenyi, K., Sreenivasan, V., Portales, A., Fernandez-Otero, M., & Marin, O. (2018). Pyramidal cell regulation of interneuron survival sculpts cortical networks. *Nature*, *557*(7707), 668-673. <https://doi.org/10.1038/s41586-018-0139-6>
- Wu, S. J., Sevier, E., Dwivedi, D., Saldi, G. A., Hairston, A., Yu, S., Abbott, L., Choi, D. H., Sherer, M., Qiu, Y., Shinde, A., Lenahan, M., Rizzo, D., Xu, Q., Barrera, I., Kumar, V., Marrero, G., Pronneke, A., Huang, S.,...Fishell, G. (2023). Cortical somatostatin interneuron subtypes form cell-type-specific circuits. *Neuron*, *111*(17), 2675-2692 e2679. <https://doi.org/10.1016/j.neuron.2023.05.032>
- Xiang, Y., Tanaka, Y., Patterson, B., Kang, Y. J., Govindaiah, G., Roselaar, N., Cakir, B., Kim, K. Y., Lombroso, A. P., Hwang, S. M., Zhong, M., Stanley, E. G., Elefanty, A. G., Naegele, J. R., Lee, S. H., Weissman, S. M., & Park, I. H. (2017). Fusion of Regionally Specified hPSC-Derived Organoids Models Human Brain Development and Interneuron Migration. *Cell Stem Cell*, *21*(3), 383-398 e387. <https://doi.org/10.1016/j.stem.2017.07.007>
- Xu, H., Jeong, H. Y., Tremblay, R., & Rudy, B. (2013). Neocortical somatostatin-expressing GABAergic interneurons disinhibit the thalamorecipient layer 4. *Neuron*, *77*(1), 155-167. <https://doi.org/10.1016/j.neuron.2012.11.004>

- Xu, Q., Guo, L., Moore, H., Waclaw, R. R., Campbell, K., & Anderson, S. A. (2010). Sonic hedgehog signaling confers ventral telencephalic progenitors with distinct cortical interneuron fates. *Neuron*, 65(3), 328-340. <https://doi.org/10.1016/j.neuron.2010.01.004>
- Xu, X., Roby, K. D., & Callaway, E. M. (2006). Mouse cortical inhibitory neuron type that coexpresses somatostatin and calretinin. *J Comp Neurol*, 499(1), 144-160. <https://doi.org/10.1002/cne.21101>
- Ye, Z., Mostajo-Radji, M. A., Brown, J. R., Rouaux, C., Tomassy, G. S., Hensch, T. K., & Arlotta, P. (2015). Instructing Perisomatic Inhibition by Direct Lineage Reprogramming of Neocortical Projection Neurons. *Neuron*, 88(3), 475-483. <https://doi.org/10.1016/j.neuron.2015.10.006>
- Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., Slukvin, II, & Thomson, J. A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318(5858), 1917-1920. <https://doi.org/10.1126/science.1151526>
- Zhang, L., Qin, Z., Ricke, K. M., Cruz, S. A., Stewart, A. F. R., & Chen, H. H. (2020). Hyperactivated PTP1B phosphatase in parvalbumin neurons alters anterior cingulate inhibitory circuits and induces autism-like behaviors. *Nat Commun*, 11(1), 1017. <https://doi.org/10.1038/s41467-020-14813-z>
- Zheng, J., Li, T., Qi, S., Qin, B., Yu, J., & Chen, G. (2022). Neuroregenerative gene therapy to treat temporal lobe epilepsy in a rat model. *Prog Neurobiol*, 208, 102198. <https://doi.org/10.1016/j.pneurobio.2021.102198>

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