The mechanism of UCoRe via Interneurons-OPCs Crosstalk: Mouse to Human Models

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Introduction

During embryogenesis, neural cells are generated and migrate to the developing cortex, guided by intricate crosstalk between different cell types that affect their migration, maturation, and integration.

Our research shows that in the embryonic mouse medial ganglionic eminence (MGE), ventral oligodendrocyte precursor cells (vOPCs) guide cortical interneuron (cINs) migration through a unique mechanism called **unidirectional contact repulsion (UCoRe)**. During UCoRe, cINs form unidirectional contacts with vOPCs, triggering changes in neuronal polarity and directing migration away from the contact point, which prevents abnormal cINs accumulation near blood vessels and ensures proper cortical placement. This process is mediated by interactions between Sema6A/B on vOPCs and PlexinA3/B2 on cINs. PlexinA3 promotes growth cone retraction (an actin-dependent process), while PlexinB2 facilitates leading process extension.

Our experiments 1/using siRNA and live imaging revealed that inhibiting PlexinA3/B2 disrupts actin dynamics and growth cone retraction in cINs, and downregulation of PlexinA3/B2 reduces RhoA activity, further impairing UCoRe. 2/UCoRe is highly conserved across mice, ferrets, and humans, with migration becoming more prolonged and complex in larger brains. 3/Notably, human ventral organoids lacking blood vessels exhibit deficient UCoRe, suggesting a critical role for vascular structures in this process.

This study will allow us to understand the cytoskeleton interactions and signal transmission facilitating between neuron-glia-vascular crosstalk.

Aims

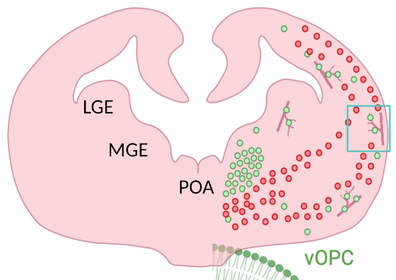
1/ Decipher the molecular mechanisms acting downstream UCoRe in INs by assessing MT and actin dynamics

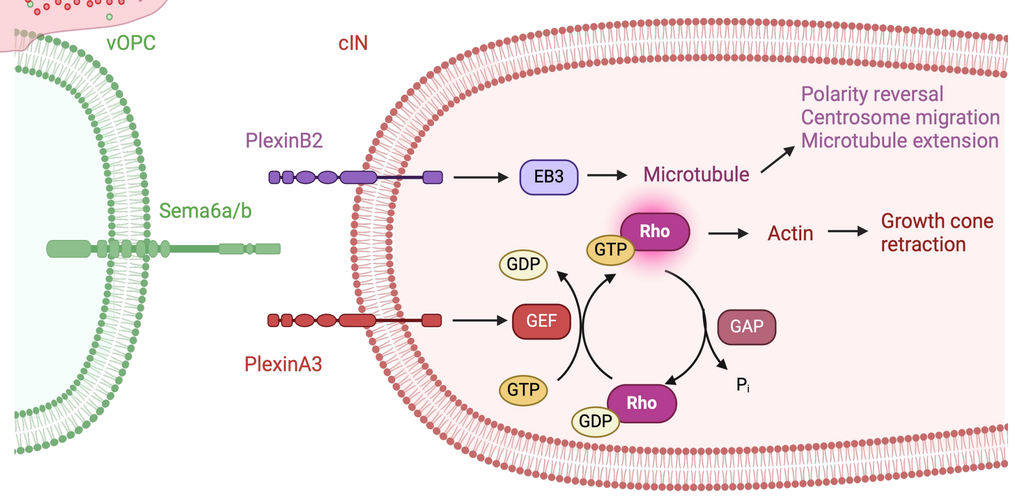
2/ Assess the interaction between vOPCs and INs across species

Conclusion

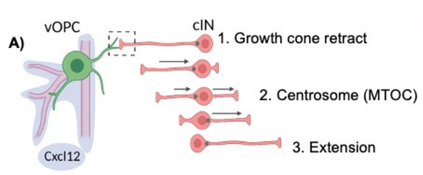
When PlexinA3/B2 was inhibited, the intensity of LifeAct and RhoA activity in the growth cone significantly decreased in cINs when touches vOPCs, resulted in defect in growth cone retraction and leading process extension.

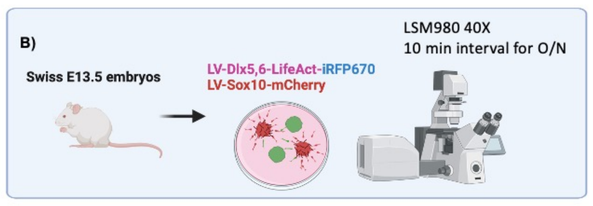
UCoRe become more protracted and complex across species. Human organoids lacking blood vessels show deficient UCoRe, highlighting the critical role of vascular in the process.

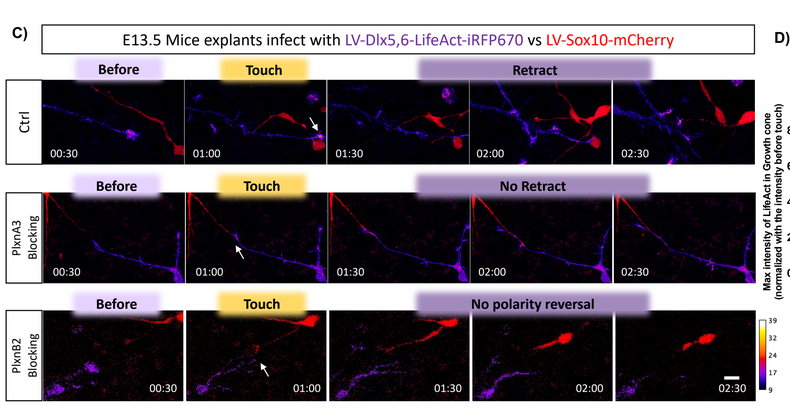


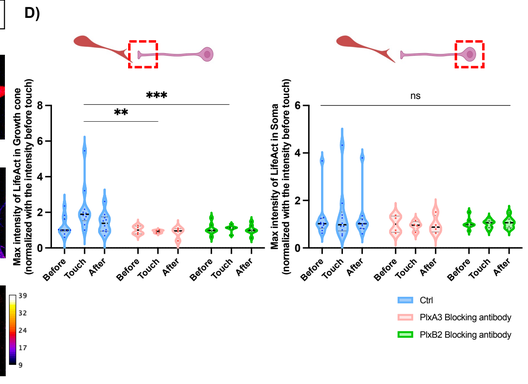


PlexinA3/B2 inhibition disrupts actin dynamics in the growth cone of cINs



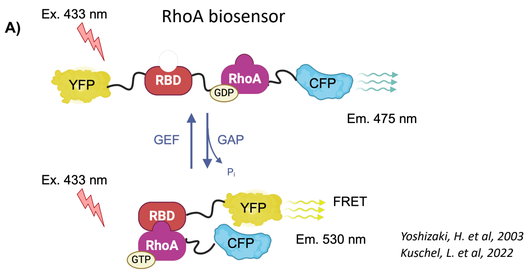




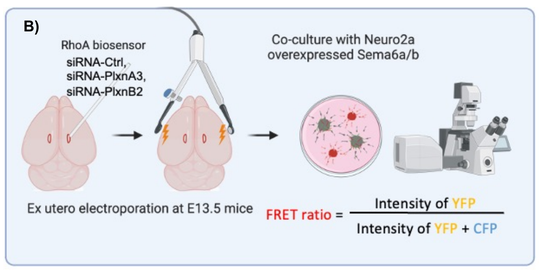


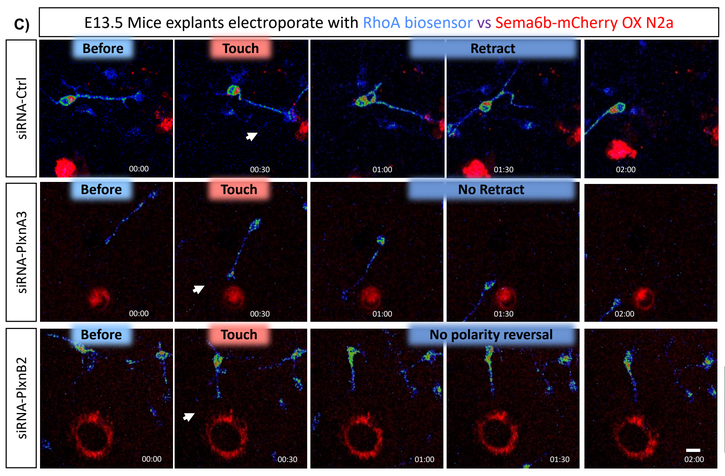
(A) Schematic representation of the steps of UCoRe between migrating cINs and vOPCs. (B) Experimental setup (C, D) The intensity of LifeAct in the growth cone increase when cINs touches vOPCs and this without changes in the cell soma. When PlexinA3/B2 was inhibited, the intensity of LifeAct in the growth cone significantly decreased in cINs when touches vOPCs, resulted in no growth cone retraction and no leading process extension.

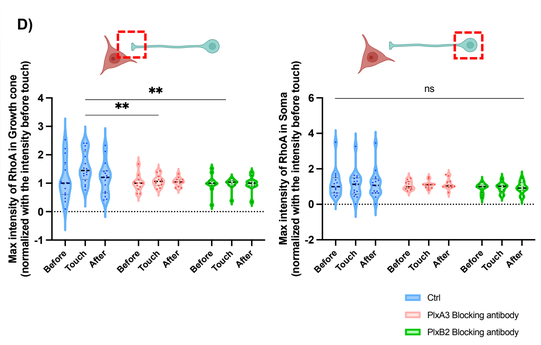
The dynamics of RhoA in cINs during UCoRe upon PlexinA3/B2 modulation



Yoshizaki, H. et al, 2003
Kuschel, L. et al, 2022

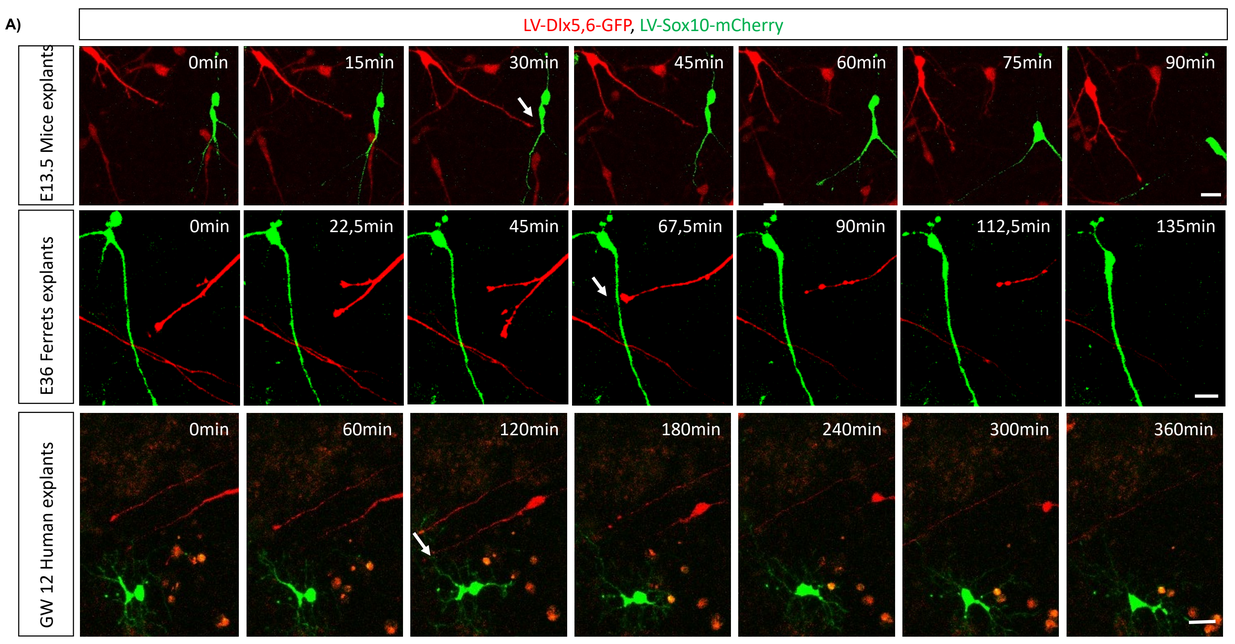


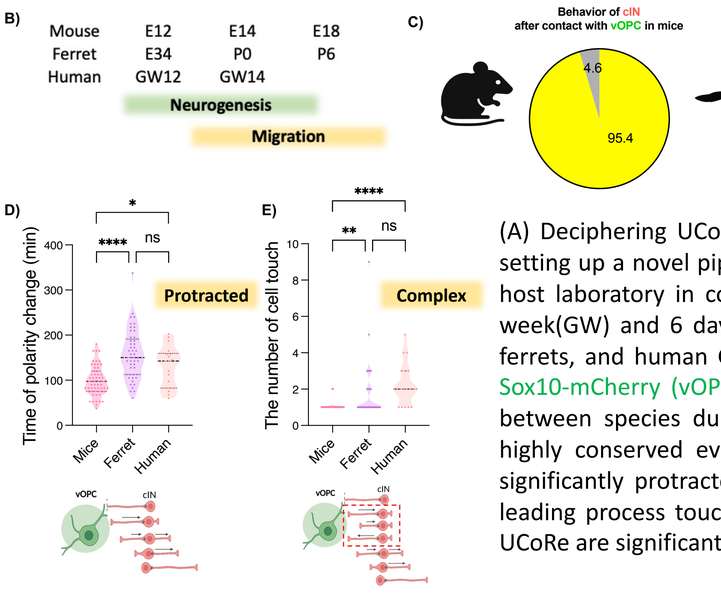




(A) Scheme of Raichu-RhoA FRET biosensor for RhoA activation mediated by GEF and GAP. (B) Experimental designusing FRET-based RhoA biosensor time-lapse recordings. (C, D) When PlexinA3 is knocked down, cINs still make contact with Sema6b-overexpressing N2a cells but fail to retract their growth cones, showing reduced RhoA intensity in the growth cone compared to siRNA-Ctrl. When PlexinB2 is knocked down, cINs still can make contact with Sema6b-overexpressing N2a but fail to induces leading process extension and with a lower RhoA intensity in the growth cone, suggesting RhoA GTPase pathway may be activated during UCoRe.

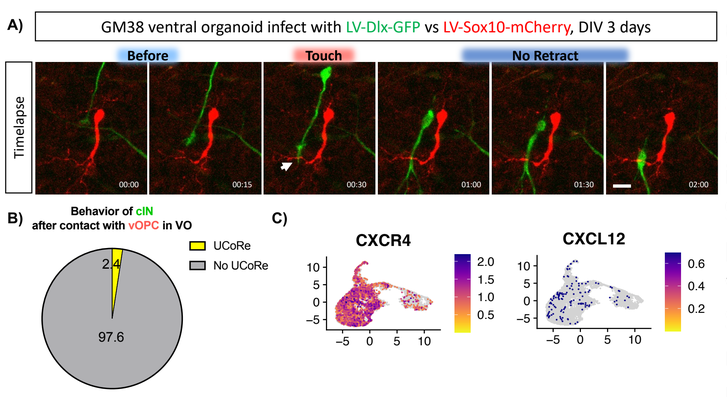
The duration and migration complexity of UCoRe increase across species





(A) Deciphering UCoRe in ferrets and human embryonic explants by setting up a novel pipeline to collect human fetal brain samples in the host laboratory in collaboration with gynecologists (< 13 gestational week(GW) and 6 days, by Belgian law). Time-lapse imaging of mice, ferrets, and human GE explants infected with lentiviruses expressing Sox10-mCherry (vOPC) and Dlx5,6-GFP (cIN). (B) Corresponding ages between species during development. (C) We found that UCoRe is highly conserved evolutionarily. (D) The time of polarity change is significantly protracted across species. (E) Moreover, the number of leading process touches between cINs and OPCs before induction of UCoRe are significantly increased across species.

cINs do not undergo UCoRe with vOPCs in human ventral organoids



(A) We tested our established lentivirus infection protocol (Dlx5,6-GFP for INs and Sox10-mCherry for OPCs) in 2-month-old GM38 ventral organoids. (B) Time-lapse imaging after 3 days *in vitro* revealed that 97.6% of INs failed to perform UCoRe, possibly due to the absence of blood vessels. (C) Our preliminary analysis from ScRNA-Seq showed that INs and OPCs do not express CXCR4 and CXCL12 in 2-month-old GM38 ventral organoids.

Acknowledgement

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