# “Mechanisms of cortical interneuron development in a mouse mutant with reduced inhibition”

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GABAergic interneurons play important roles in cortical function and their loss/ dysfunction is implicated in severe disorders (schizophrenia, epilepsy). We have demonstrated the unique and diverse roles of the RhoGTPases Rac1 and 3 in interneuron progenitors and their morphology in transgenic animals where Rac1 and Rac1/3 were ablated specifically in cortical interneurons (CINs). In the Rac1 mutant, progenitors delay their cell cycle exit resulting in a 50% decrease in CINs and an imbalance of excitation/inhibition in cortical circuits. In the Rac1/3 mutant, there are additional cytoskeletal defects resulting in an 80% decrease in CINs. Both lines die from epileptic seizures. Our data suggests that proper levels of inhibition early postnatally, is critical for the development of synaptic properties and plasticity of the prefrontal cortex (PFC). We report on the maturation of early PFC circuits in a state of reduced inhibition and on the cellular/molecular mechanisms guiding these mutant interneurons to the cortex. These data should contribute to the understanding of CIN function, especially since several preclinical models of CIN-based cell therapies are being designed.

## Ablation of Rac1 and Rac3 from GABAergic interneurons leads to delayed migration partially due to morphological defects

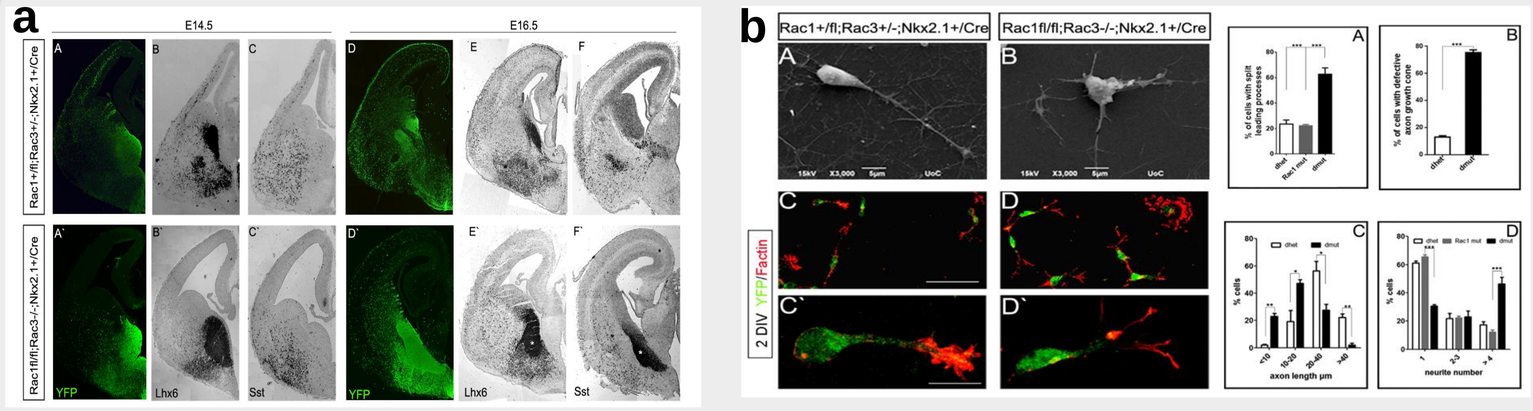



**Figure 1: a.** Coronal sections from the forebrain of developing embryos, P5 and P15 brains. At E14.5 (a;A-C:A’-C’), E16.5 (a;E,F:E’,F’`), P5 brains (b;A,B:A’,B’) and P15 (b;C,D:C’,D’) are analysed for specific markers of GABAergic interneurons. **b.** MGE-derived double mutant cells revealed splitting of the leading process (b;A-D: C’,D’, c;A), absence of lamellipodia (b;C,D:C’,D’, c;B), increase in neurite number (b;E,F:E’,F’, c;D) and reduction of axon length (c;C).

## The motility of Rac1/3 deficient MGE-derived cells is significantly reduced

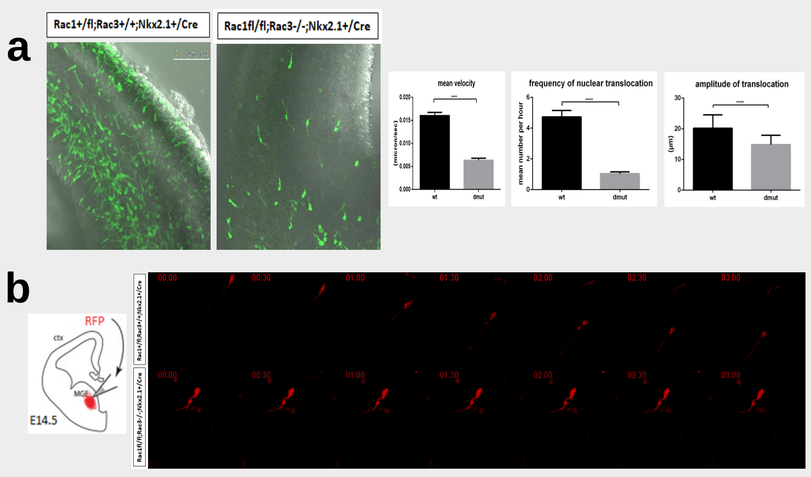



**Figure 2. a.** Live imaging of cortical slices from E14.5 control and double mutants (3 min. time interval).Mean velocity,( wt cells n=273, dmut cells n=118),frequency of nuclear translocation( wt cells n=80, dmut cells n=68)amplitude of translocation (wt cells n=45, dmut cells n=41).are shown in different graphs. **b.** focal electroporation with RFP on cortical E14.5 organotypic slices (Live imaging, 24hrs after plating; 3 min time interval).

## Outgrowth of principal neurite and microtubule stability are affected in Rac1/3 deficient interneurons

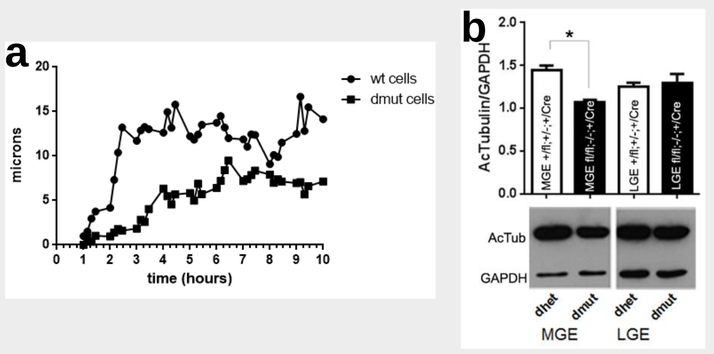



**Figure 5. a.** MGE-derived interneurons were cultured on matrigel. The distance of the tip of the proximal neurite from the axon initial segment over time is plotted. Live imaging with confocal microscope, 1h after plating for 10hrs. time interval: 3min. Average values (wt cells n=10, dmut cells n=10) **b.** the amount of Ac-Tubulin is reduced in the Rac1/Rac3 deficient MGE-derived cells

## Rac1/3 deficient interneurons show defects in Golgi Complex and centrosome positioning

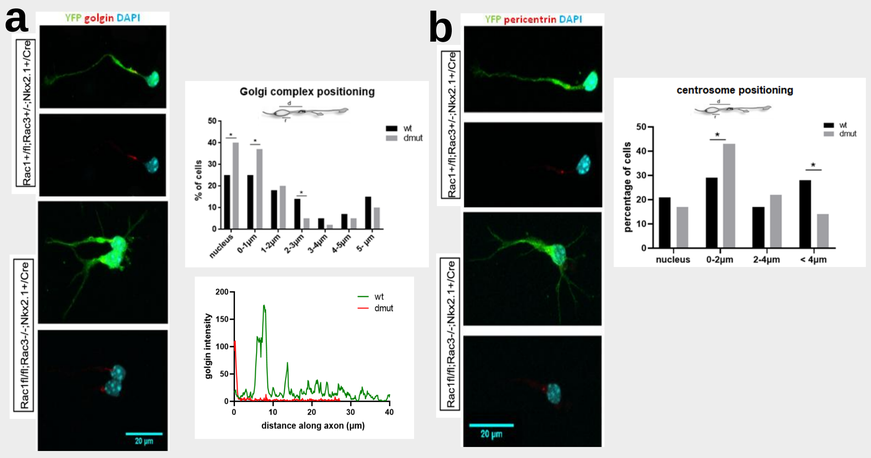



**Figure 3**. MGE-derived interneurons cultured 3 DIV. **a**. distance of the Golgi complex position from the nucleus (d-r), Golgin intensity plot **b**. distance of the centrosome position from the nucleus (d-r), (wt cells n=120, dmut cells n=120)

## Gene expression is altered in Rac1/3 deficient MGE-derived interneurons

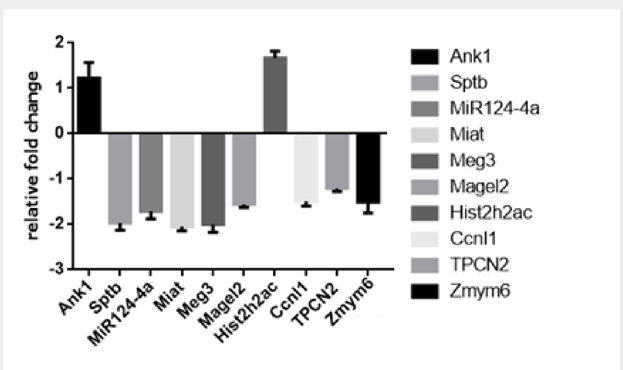



**Figure 6**. RT-qPCR in MGE-derived double mutant cells from E13.5 embryos normalized to control MGE-derived cells (N=3, error bars represent SEM) for a subset of genes that showing altered expression in RNA seq analysis (Gapdh was used as an internal control)

## Decreased number of interneurons in Rac1/Rac3 mt in mPFC, at P10

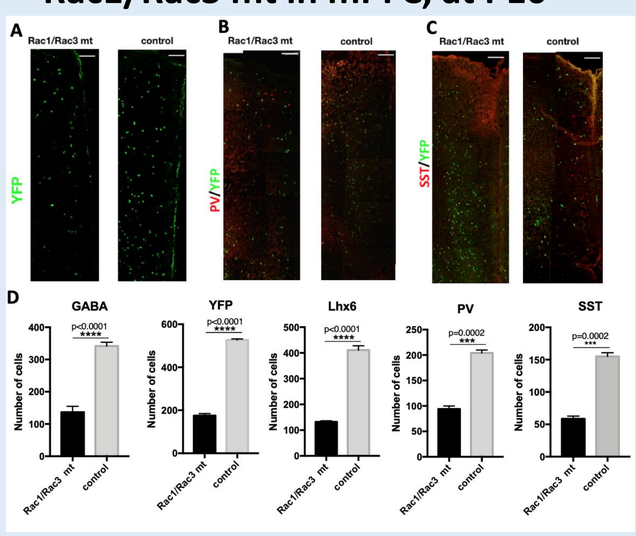



**Figure 1:** Coronal sections from control and Rac1/Rac3 mt mice immunostained using anti-GFP (**A**), anti-PV and anti-GFP (**B**) and anti-SST and anti-GFP antibodies (**C**). PV: parvalbumin, SST: somatostatin. Scale bar 150µm. (**D**) Graphs showing that the number of YFP-, Lhx6-, PV-, SST- and GABA positive interneurons was reduced in the Rac1/Rac3 mt mice compared to control mice (t-test, $p < 0.0102$, $n = 5$ slices from three control and $n = 4$ slices from three Rac1/Rac3 mt mice).

## Rac1/Rac3 mt exhibits decreased basal synaptic transmission and increase spontaneous activity in mPFC, at P10

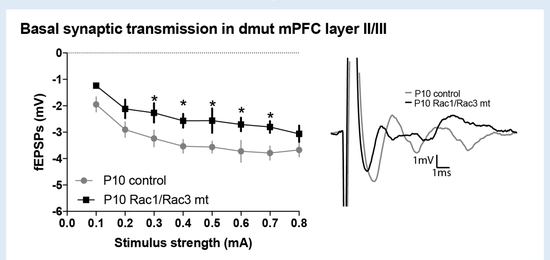
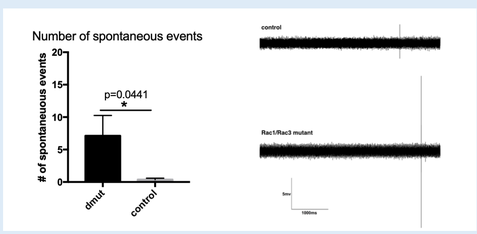



**Figure 2:** Representative traces from LFP recordings on slices from mPFC of WT and Rac1/Rac3 mt, at P10 (right). Graph (left) comparing the fEPSPs amplitude between WT and Rac1/Rac3 mt mice, at P10 in mPFC. Two-way ANOVA analyses of evoked fEPSPs revealed a significant effect of stimulus strength ($F_{(7, 65)} = 7.42$, $p<0.0001$) and experimental groups ($F(1, 65) = 32.27$, $p<0.0001$). Post-hoc analysis showed that the fEPSPs amplitude was significantly decreased in Rac1/Rac3 mt compared the WT mice at P10 in mPFC (LSD test, *p= 0.02632), (n=6-7 brain slices from 3-4 mice).




Representative voltage traces from spontaneous activity recordings from control and Rac1/Rac3 mt brain slices (right). Graph showing the number of spontaneous events that was significantly greater in Rac1/Rac3 mt compared to control brain slices (*t*-test, p=0.0441, n = 5 slices from three control and *n* = 4 slices from three Rac1/Rac3 mt mice).

**Ablation of Rac1 and Rac3 from GABAergic interneurons leads to:**

- morphological defects resulting in reduced motility and delayed migration
- defective Golgi and centrosome positioning
- an 80% reduction of MGE-derived GABAergic interneurons in the cortex
- the loss of interneurons results in a significant decrease in basal synaptic transmission early postnatal period
- A significantly increased spontaneous activity in Rac1/Rac3 mt
- candidate effectors (in progress) revealed by RNAseq analysis (cytoskeletal effectors, histones, microRNAs)

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