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The role of small RhoGTPases Rac1 & Rac3 in cortical GABAergic interneuron development

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Abstract

GABAergic interneurons play important roles in cortical function and provide the main source of inhibition to cortical microcircuits. Impaired interneuronal function results in severe neurodevelopmental disorders such as schizophrenia, epilepsy and autism. Although recent studies have uncovered some of the molecular components underlying interneuron development, including the cellular and molecular mechanisms guiding their migration to the cortex, the intracellular components involved are still unknown. Rac proteins, the members of the Rac subfamily of Rho GTPases, have been implicated in various cellular processes such as cell cycle dynamics, axonogenesis and migration. We are interested in elucidating the roles of Rac1 and Rac3 specifically in MGE-derived interneurons, a population that comprises the majority of all cortical interneurons. We have used conditional Rac1 (Rac1fl/fl) deficient mice using the Cre/loxP technology combined with Rac3 (Rac3-/-) null mice. Recent data from our team revealed that 50% of MGE-derived GABAergic interneurons fail to migrate and populate the postnatal cortex of Rac1 deficient mice. We also pointed out the role Rac1 activity in interneuron proliferating, correlating Rac1 with cell cycle progression (Vidaki et al., 2011). Our data revealed that in the absence of Rac1 and Rac3 proteins, embryonic migration of MGE derived interneurons is affected. In addition postnatally, MGE-derived interneurons missing both Rac1 and Rac3 proteins show an even more severe defect than Rac1 ablation only (80% of cortical interneurons are absent, especially the parvalbumin and somatostatin subpopulations) and the mice die even earlier than the Rac1 single mutants. Our aim is to decipher the molecular mechanisms underlying the observed defects in the mice lacking both Racs from their cortical interneurons.

Keywords: cortical development, inhibitory interneurons, Rac1 and Rac3 Rho-GTPase

Introduction

1. Interneurons

The proper functioning of the adult cerebral cortex requires a network of excitatory and inhibitory stimuli primarily produced by projection neurons (using glutamate as a neurotransmitter) and interneurons (using γ-aminobutyric acid (GABA) as a neurotransmitter). Loss or dysfunction of GABAergic interneurons is associated with severe disorders such as schizophrenia and epilepsy (Cobos et al. 2005; Butt et al. 2008). Evidence suggest that disruption of the excitatory–inhibitory balance maintained by pyramidal cells and interneurons is linked to the etiology of several neurological disorders, such as autism, Rett Syndrome, epilepsy, schizophrenia (Rubenstein & Merzenich, 2003; Dani et al., 2005; Levitt, 2005; Lewis et al., 2005). Consequently, disruption of GABAergic inputs in pyramidal cells might point out a common pathophysiological mechanism underlying multiple neuropsychiatric conditions. Extensive studies over the past decade have provided a comprehensive view of excitatory neurogenesis in the developing neocortex. In contrast, our knowledge of neocortical interneuron generation remains incomplete.

Interneurons constitute the 20–30% of the cortical neuronal population and have smooth or sparsely spiny dendrites and locally projecting axons. Interneurons have a vital role in modulating cortical output and plasticity (Whittington et al., 2003; Wang, X., et al., 2004). Interestingly, the influence of GABAergic interneurons on pyramidal cells is largely dependent on the subcellular location of their inputs, which varies among different interneuron subtypes. Cortical interneurons have also been implicated in developmental processes, including the regulation of neuronal migration during corticogenesis and the development of cortical circuitry (Heck, et al., 2007; Owens, D. F. & Kriegstein, A. R., 2002).

Today it is largely accepted that distinct types of interneurons exist but the exact subtype number is not known; following the recommendation of the Petilla terminology (Petilla Interneuron Nomenclature Group et al., 2008), they are defined by a consideration of neurochemical, anatomical and electrophysiological characteristics. Depending on above criteria of classification, there is a conservative grouping of GABAergic interneurons into four major classes:

(1) **fast-spiking, PV-containing** basket ;
(2) **somatostatin (SST)-containing** interneurons, which typically display intrinsic burst spiking or adapting non-fast-spiking electrophysiological profiles and many of which have long axons that extend into layer I;
(3) Rapidly adapting interneurons with bipolar or double-bouquet morphologies, which frequently express **calretinin (CR)** and ⁄ or **vasointestinal peptide (VIP),** and
(4) Rapidly adapting interneurons with multipolar morphologies and that express **neuropeptide Y (NPY)** and ⁄ or reelin, but not SST.

A recent study (Rudy et al., 2011) has presented a new classification, in which nearly 100% of all neocortical GABAergic neurons belong to one of three groups defined by the expression of **parvalbumin (PV), somatostatin (SST),** and the ionotropic serotonin receptor **5HT3a (5HT3aR)**. Each group consists of several subgroups. In turn, each subgroup consists of several functionally distinct types or classes of interneurons, most of which are still poorly defined ***(Figure 1).*** The past years, studies have tried to uncover some of the molecular

components underlying the generation of this diversity (Marin and Rubenstein, 2003; Métin et al., 2006).

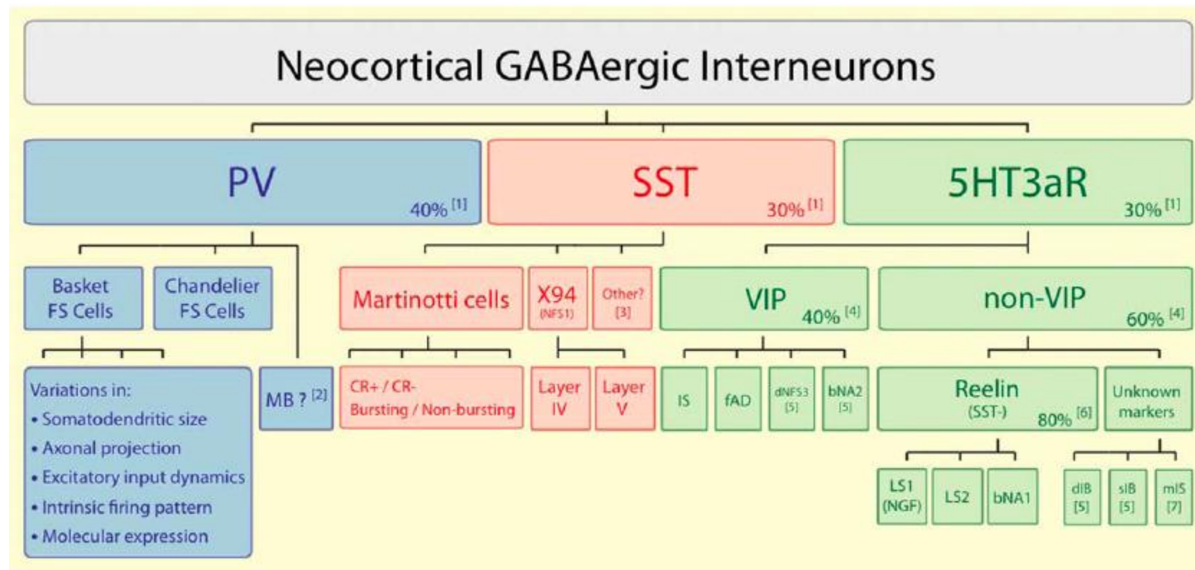

Figure 1. **The most recently classification of neocortical GABAergic interneurons** (Rudy et al., 2011).

1.2. Origin of Interneurons

The specification of the interneuronal population in the distinct subtypes requires the coordination of diverse molecular cues deriving from the place of interneuronal origin to their final destination in the cortex. The cellular and molecular mechanisms guiding interneurons from their place of origin to the cortex have recently started to be elucidated (Marin and Rubenstein, 2001; Marin and Rubenstein, 2003; Métin et al., 2006; Butt et al. 2008). Fate-mapping experiments both in vivo and in vitro have demonstrated that the ventral forebrain is the primary source of cortical interneurons in rodents (Corbin et al., 2001; Marin and Rubenstein, 2001; Wonders and Anderson, 2006). Projection neurons are born from radial glial cells in the ventricular zone and migrate radially on radial glial fibers towards the pial surface. Interneurons expressing GABA, originate from the embryonic subpallium structures, the LGE (lateral ganglionic eminence), MGE (medial ganglionic eminence), and preoptic area (POA), and migrate rostrally into the olfactory bulb or tangentially the cortex ***(Figure 2A)***. The GABAergic interneurons are first specified in the medial ganglionic eminence (MGE) of the ventral telencephalon (subpallium), and only subsequently invade the cerebral cortex through a long-distance migration using two well-defined tangential routes located in the marginal zone (MZ) and the subventricular zone/intermediate zone (SVZ/IZ) (Marin and Rubenstein, 2001; Kriegstein and Noctor, 2004;) ***(Figure 2B).*** In contrast to the multiplicity of extracellular signals, the intracellular proteins that mediate the response to these cues are unknown (Martini et al., 2009).

The MGE is the origin of about 50-60% of the population of cortical interneurons in the mouse (Miyoshi et al., 2010). The majority of the MGE cells express Nkx2.1 (Sussel et al., 1999). By contrast, the dorsal MGE expresses Nkx6.2 and Gli1 and is partially Nkx2.1 negative (Fogarty et al., 2007; Rallu et al., 2002b; Wonders et al., 2008). Nkx2.1 null mice have an atrophied MGE and show a drastic reduction of cortical interneurons at birth (Sussel et al., 1999). It has recently been shown that Nkx2.1 is upstream of the LIM-homeobox

transcription factor Lhx6. Nkx2.1 directly activates Lhx6 and specifies interneuronal fate (Liodis et al., 2007; Du et al., 2008). MGE-derived progenitors start to express Lhx6 as soon as they leave the ventricular zone (Grigoriou et al., 1998). Lhx6 expression persists through adulthood in most parvalbumin (PVA)- and somatostatin (SST)-expressing cortical interneurons (Cobos et al., 2005; Du et al., 2008; Fogarty et al., 2007; Lavdas et al., 1999; Liodis et al., 2007) ***(Figure 3).***

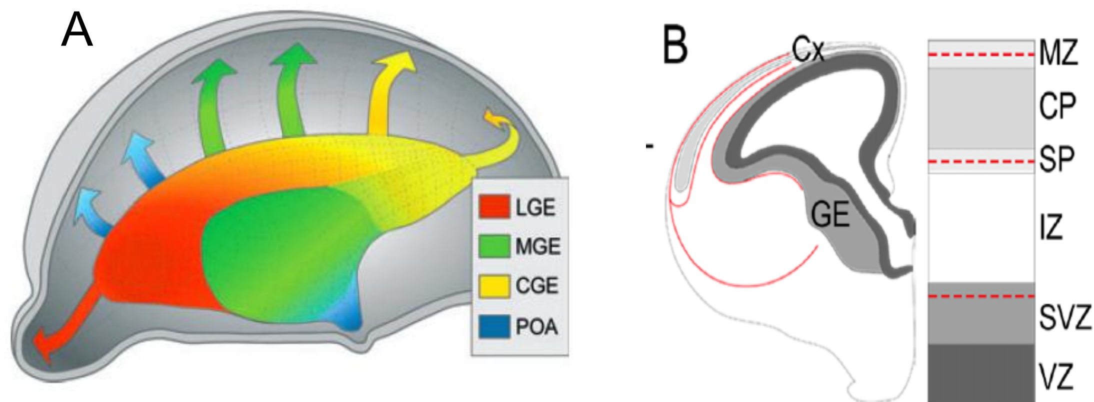

Figure 2. **Migration pathways of cortical interneuron subgroups from the ventral telencephalon. A)** Cortical interneurons are born in the subpallium and migrate tangentially to the cortex. The schema represents an E13.5 embryo brain hemisection. The arrows show representative migratory routes. POA-derived interneurons have a bias to invade the cortex through its rostral region, while CGE-derived interneurons primarily reach the cortex by its caudal pole. (Gelman & Marin, 2010). **B)** Red lines show the laminar position of each migrating interneuron stream at E15.5 (Faux et al., 2010).

Lhx6 loss of function analysis has shown that it is required for the normal specification and migration of MGE-derived GABAergic neurons. Lhx6 null animals exhibit a loss of PV and SST interneurons in the neocortex and hippocampus (Liodis et al., 2007). Hence, the MGE appears to be the sole source of three of the most prominent interneuron subtypes: the basket, chandelier, and Martinotti cells.

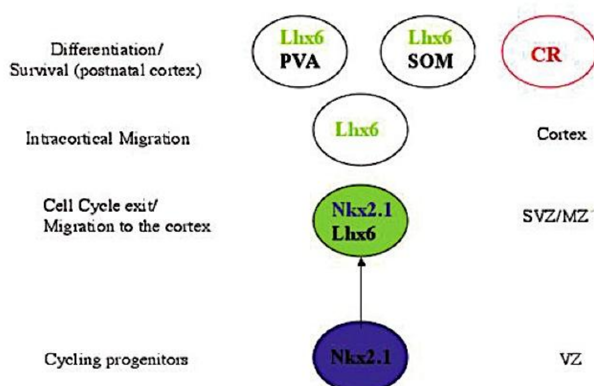

Figure 3. **Lhx6 is expressed in tangentially migrating GABAergic interneurons**. Nkx2.1 directly activates Lhx6. Lhx6 is required for the normal pattern of tangential migration of GABAergic interneuron progenitors and for their correct distribution in the cortical layers of postnatal animals. Also, Lhx6 is preferentially expressed in the Pv- and Sst-expressing subpopulations of cortical interneurons and is required in vivo for their specification (by M. Dexana).

In addition to the MGE, the CGE is the other main source of cortical interneurons. Recent data suggest that CGE may produce between 30 and 40% of all cortical interneurons (Miyoshi et al., 2010; Lee et al., 2010; Rudy et al., 2011). A proportion of interneurons (about 8–10%) may also derive from a third source, the embryonic POA (Gelman et al., 2009). Although several studies have indicated that any LGE contribution to cortical interneurons is far smaller than that of the MGE (Anderson, S. A et al., 2001; Wichterle, H., et al., 2001). Based on the latest publications, the LGE can be subdivided into a ventral part that produces striatal projection neurons (Olsson et al., 1998; Stenman et al., 2003a) and a dorsal portion that gives rise to the postnatal SVZ, as well as to olfactory bulb interneurons (Stenman et al., 2003a).

2. Rho-GTPases

The Ras superfamily of small GTPases, consisting of almost 200 proteins, can be subclassified into six families: Rho, Ras, Rab, Arf, Sar, and Ran (Colicelli 2004). The Rho GTPases act as molecular switches to control signaling events by alternating between GTP- and GDP-bound states. (Etienne-Manneville S. & Hall A., 2002). The Rho GTPases are activated by the guanine nucleotide exchange factors (GEFs) that replace the GDP with GTP. The GTPase-activating proteins (GAPs) act as the negative regulator of the molecular switch by catalyzing the hydrolysis of GTP to GDP. The Rho GTPases are normally sequestered at the cytoplasm by RhoGDI (guanine dissociation inhibitor) until the cell is stimulated. The amino (N-) termini of Rho GTPases are well conserved while the carboxy (C-) termini vary and may be associated with different functions (Chen Z., et al., 2000; Didsbury JR, et al., 1990). There are effector proteins which are downstream of the Rho GTPases. They bind to and are activated by the GTP-bound form of the GTPases. Three of the most well studied Rho GTPases are RhoA, Cdc42 and Rac1 which have been reported to be antagonistic to one another. Generally, RhoA stimulates neurite retraction while Rac and Cdc24 promote neurite outgrowth (Koh CG. ,2007) ***(Figure 4).***

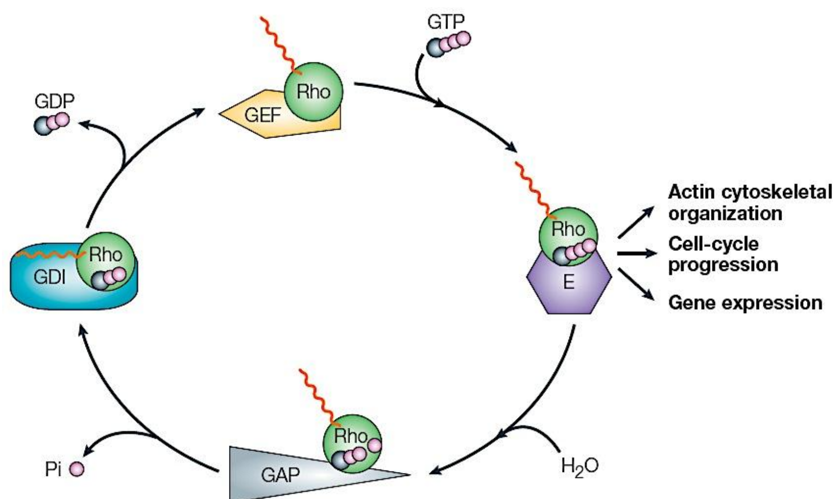

Figure 4. **Regulating Rho-GTPase activity. (Rossman et al., 2005).**

Proteins of the Rac subfamily of Rho-GTPases are involved in many cellular functions, such as regulation of actin dynamics, cell cycle progression, establishment of polarity, and

axonogenesis (Jaffe and Hall 2005; Watabe-Uchida et al. 2006; Koh 2007; Chen L, et al., 2007). This subfamily consists of 3 members: Rac1, an ubiquitously expressed protein; Rac2, expressed mostly in the hematopoietic system; and Rac3, which is highly enriched in the nervous system (Malosio et al. 1997). There are more studies about the Rac1 involvement in various systems but lesser for Rac2 and Rac3 roles.

2.1. Rac proteins as actin cytoskeleton regulators

In vitro experiments have showed that Racs promote neurite production in neuroblastoma cells (Sarner et al. 2000) but also the axon elongation and guidance in PC12 cells (derived from a transplantable rat pheochromocytoma.) (Yoshizawa et al. ,2005). Moreover, experiments in primary cultures from hippocampal neurons have demonstrated that Rac1 leads the induction and elongation of neuroaxons (Schwamborn et al., 2004). In developmental dorsal telencephalon, Rac1 colocalized and interacted with cyclin-dependent kinase 5, cdk5, essential for neuronal migration and for the laminar configuration of the cerebral cortex of pyramidal neurons (Nikolic et al., 1998). The suppression of Rac1 activity caused loss of the leading process of pyramidal neurons in the dorsal telencephalon, (Kawauchi, et al., 2003). Also, Rac1 has a critical role in axon guidance and in acquisition of migratory competency during differentiation of the progenitors for the ventral telencephalon-derived interneurons (Chen et al., 2007).

The Rac proteins are well known for their ability to stimulate actin polymerization at the plasma membrane, forming lamellipodia. The effector proteins downstream of Rac1 involved in lamellipodia formation are mainly the WAVE subfamily of the WASP proteins in epithelia cells (Miki H, et al., 1998,2000). More particularly, Rac1 acting through WAVE and Arp2/3 regulates neuronal polarization and lamellipodia formation (Tahirovic et al. 2010) and is required for promoting actin polymerization at the front of migrating cells, thus pushing forward the leading edge membrane (Gardiner et al. 2002; Itoh et al. 2002) ***(Figure 5).***

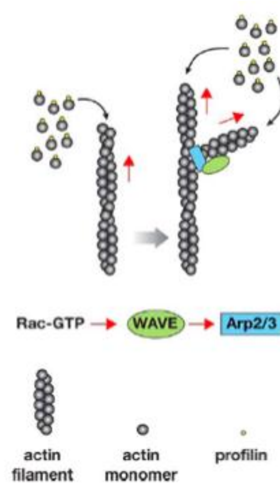

Figure 5. **Rac GTPase regulates the actin polymerization.** Rac activate Arp2/3 via WAVE (aWASP family protein) to initiate a branched filament network (Jaffe & Hall, 2005).

Rac1 is also required for microtubule assembly during cell movement, via IQGAP1/CLIP170 (Fukata et al. 2002). Additionally, the activation of Arp2/3 by Rac leads to activation of cortactin, an important nucleation-promoting factor of Arp2/3 that has an essential role in

many actin remodeling processes such as migration and axon guidance (Ammer and Weed 2008). Other studies have noted the inhibitory action of Rac through the inactivation of the actin- depolymerizing factor cofilin. Phosphorylation of cofilin leads to inactivation and occurs primarily through LIM kinases (LIMK), which in turn are activated by the PAK family of Rac/Cdc42-dependent kinases (Ng and Luo,2004) ***(Figure 6).***

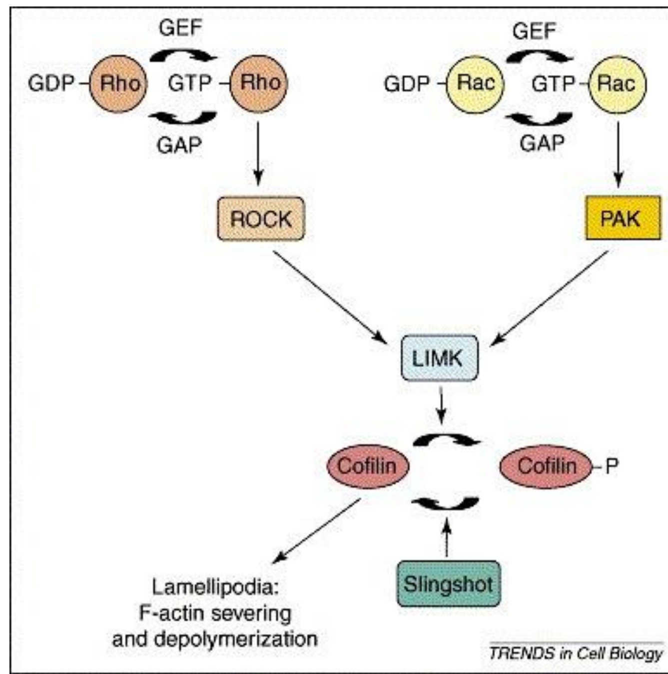

Figure 6. **Regulation of cofilin by Rho and Rac.** Rho and Rac are activated by exchange of GDP to GTP, catalyzed by guanine nucleotide exchange factors (GEFs). They are inactivated by GTP hydrolysis, catalyzed by GTPase-activating proteins (GAPs). In their active, GTP-bound conformation, Rho-GTP binds to and activates ROCK serine/threonine kinases, whereas Rac-GTP binds to and activates PAK serine/threonine kinases. ROCKs and PAKs can then phosphorylate and activate LIMKs, which in turn phosphorylate and inactivate cofilin. Cofilin is dephosphorylated by the phosphatase Slingshot. Unphosphorylated cofilin stimulates severing and depolymerization of filamentous actin (F-actin) in lamellipodia (Ridley, 2006).

2.2. Rac proteins as Cell cycle regulators

A more enigmatic aspect of Rac protein function is that of cell cycle regulation. It is known that the function of Rac1 may be different, depending on the specific cell type and tissue (Jaffe and Hall 2005; Fuchs et al., 2009). Recent data showed that Rac1 deletion in the VZ of the entire telencephalon by the Foxg1 Cre line resulted in accelerated cell-cycle exit and increased apoptosis of the telencephalic VZ progenitors during early corticogenesis . It has been suggested that Rac1 regulates self-renewal, survival, and differentiation of telencephalic neural progenitors, and that dysfunctions of Rac1 may lead to primary microcephaly (Chen et al. 2009).

On the other hand, our data show that ablation of Rac1 in the MGE (using the Nkx2.1 Cre line) is causing progenitors to remain longer in cell cycle with no accompanying effect on apoptosis. Also, this cell cycle delay could be through potential regulation of cyclin D protein levels, while Rb phosphorylation Rac1 could facilitate the transition from the G1 phase of an actively cycling condition to the postmitotic/ migrating phase (Vidaki et al., 2011).

Rac 1 and Cdc42 control the cell cycle of neural crest stem cells and Cdc42 or Rac1 inactivation reduces self-renewal and increases cell-cycle exit (Fuchs et al., 2009). older studies have shown that suppression of Rac1 and Cdc42 activity led to cell cycle progression blockage via G1 inhibition (Olson et al.,1995; Yamamoto et al., 1995). It is clear that Rac can regulate the transcription of Cyclin D1 a key cell cycle regulator that activates the cyclin-dependent kinase CDK4 and CDK6 during the G1 phase of cell cycle. There have been several reports that Rac and Cdc42 stimulate cyclin D1 transcription when ectopically expressed in cells, and in one case at least, this was mediated by NFκB (Joyce et al. 1999, Westwick et al.1997). Also, cell cycle progression is accomplished via tightly regulated expression of Rho, Rac1, and ERK, which play an important role in the balanced expression of cyclin-dependent kinases (Welsh et al. 2001). Although several studies have been carried out to reveal the role of Rho-GTPases in multiple systems, the molecular details of their role in the development of the brain is largely unknown

Aim of Study

Recent data from our lab has shown that small Rho-GTPases, Rac1 and Rac3 play a role as intracellular mediators of cortical interneuron development. Mice lacking these regulatory proteins exhibit a severe reduction of GABAergic interneurons that reach the cortex (Vidaki et al., 2011, Tivodar et al., in preparation). We plan to investigate the molecular mechanism through which Rac1 and Rac3 GTPases contribute to interneuron development and determine whether the absence of Rac3 in the Rac1-deficient interneurons has an additive effect. Moreover, we plan to fully characterize Rac1 and Rac3 deficient mice that exhibit a strong reduction in GABAergic interneurons thus generating new models to study neurodevelopmental disorders. My work focused on the postnatal analysis of mice mutant for both Racs.

Materials and Methods

Generation of Nkx2.1-Cre-directed conditional Rac1 deficient mice and Rac3 knock out: Animals carrying a floxed allele of Rac1 (Rac1fl/fl) (the 4th and 5th exon of the Rac1 gene are flanked with loxP sites, Walmsley et al., 2003) were crossed to Nkx2.1Tg(Cre) mice (Nkx2.1 transgenic Cre, Fogarty et al., 2007), in order to generate the Rac1fl/fl;Nkx2.1Tg(Cre) genotype. To obtained double mutant animals the Rac1 +/fl;Nkx2.1Tg(Cre) line was crossed with Rac3 KO line (Corbetta et al., 2005). The ROSA26fl-STOP-fl-YFP allele was also inserted as an independent marker (Srinivas et al., 2001), to allow visualization of the Rac1/3 mutant neurons, via YFP expression. Rac1fl/fl;Nkx2.1Tg(Cre);Rac3-/-;R26R-YFP+/- and Rac1+/fl;Nkx2.1Tg(Cre) ;Rac3+/-;R26R-YFP+/- animals will be referred to as Rac1fl/fl;Nkx2.1+/Cre;Rac3-/- and Rac1+/fl;Nkx2.1+/Cre;Rac3+/- respectively in Materials, Figures and legends. The genotyping was performed by PCR, using specific primers for:

Rac1

1-GTTGAAGGTGCTAGCTTGGGAACTG

2-GAAGGAAGAAGAAGCTGACTCCCATC

3-CAGCCACAGGCAATGACAGATGTTC

YFP
1-GCTCTGAGTTGTTATCAGTAAGG
2-GCGAAGAGTTTGTCCTCAACC
3-GGAGCGGGAGAAATGGATATG
Nkx2.1-Cre
1-GTCCACCATGGTGCCCAAGAAGAAG
2-GCCTGAATTCTCAGTCCCCATCTTCGAGC
Rac3
1-CATTTCTGTGGCGTCGCCAAC
2-CACGCGGCCGAGCTGTGGTG
3-TTGCTGGTGTCCAGACCAAT
For timed pregnancies, the day of the vaginal plug was designated as embryonic day E0.5 and the day of birth was considered as P0.
The mice were available in the IMBB animal facility
Immunohistochemistry: For immunohistochemistry, embryonic brains were dissected in PBS and fixed in 4% PFA overnight, whereas adult animals were first perfused with 4% PFA, 25% glutaraldehyde and then the brains were dissected and immersed in the same fixative for 2 hours. Following fixation, the tissues were cryoprotected by infiltration in 30% sucrose in PBS and 10μm cryostat sections were collected on superfrost slides. For immunostaining, the slides were washed in 0.1%Triton X-100 in PBS (PBT), then blocked in 1%FBS, 0.1%BSA in PBT for 1hr at RT and subsequently incubated with primary antibodies, diluted in blocking solution, for 18 hrs at 4oC. Following that, the slides were washed in PBS (3 washes**,** 10 min each) and incubated with the secondary antibody, diluted in blocking solution, for 2 hrs at RT. After another 3 washes (10 min each) the slides were mounted, using MOWIOL (Calbiochem, Darmstadt, Germany). Primary antibodies used: mouse monoclonal anti-GFP (Invitrogen, San Diego, CA, 1:500), rabbit polyclonal anti-GFP (Minotech biotechnology, Heraklion, Greece, 1:5000), rat monoclonal anti-GFP (Nacalai Tesque, Kyoto, Japan, 1:500), rabbit polyclonal anti-Lhx6 (Lavdas et al, 1999, 1:200), rabbit polyclonal anti-GABA (Sigma, Saint Louis, MI, 1:1000), mouse monoclonal anti-parvalbumin (PV) (Chemicon, Temecula, CA; 1:1000), rabbit polyclonal anti-calretinin (CR) (Swant, Bellinzona, Switzerland, 1:1000), rat monoclonal anti-somatostatin (Som) (Abcam, Cambridge, MA, 1:500). Secondary antibodies used: goat anti-mouse-Alexa Fluor-488, -555, or -633, goat anti-rabbitAlexa Fluor-488, -555, or -633 and goat anti-rat-Alexa Fluor-488, -555, or -633 (all from Molecular Probes, Eugene, OR, all 1:800).
RNA In Situ Hybridization: Nonradioactive in situ hybridization on fixed cryostat sections was performed as described previously (Denaxa et al., 2001; Kyriakopoulou et al., 2002). Riboprobes used, were specific for Lhx6 (Grigoriou et al., 1998), NPY , Somatostatin (Liodis et al., 2007), Rac3 Ror beta, Cux2 and ER81 .
Nissl staining: Air dry cryosections. Place slides directly into 1:1 alcohol/chloroform overnight and then rehydrate through 100% and 95 % alcohol to distilled water. Stain in 0.1% cresyl violet solution for 5-10 minutes. Notes: Staining in warmed cresyl violet solution (warm up in 37-50 ºC oven). It is particularly beneficial for thicker (30 um) sections. Rinse quickly in distilled water. Differentiate in 95% ethyl alcohol for 2-30 minutes and check microscopically for best result. Dehydrate in 100% alcohol 2x5 min. Clear in xylene 2x5 min. Mount with permanent mounting medium.
Quantification and Statistical analysis: For quantification of GABAergic interneurons and the different inteneuronal subpopulations markers in P5 and P15 brains, at least 3 pairs of

littermate animals were used (Rac1+/fl;Nkx2.1+/Cre;Rac3+/- vs Rac1fl/fl;Nkx2.1+/Cre;Rac3-/-). For each pair, four sections corresponding to distinct bregmata along the rostrocaudal axis (-1.94 & 2.) were selected, all including the barrel cortex field. Using appropriate molecular markers (Lhx6, PV, CR, somatostatin, YFP, or GABA), images were obtained with a confocal microscope (Leica TCS SP2, Leica, Nussloch, Germany), cells in the entire barrel field were counted and an average rostrocaudal number was calculated for the interneuron subpopulations of Rac1+/fl;Nkx2.1+/Cre:Rac3+/- and Rac1fl/fl;Nkx2.1+/Cre;Rac3-/- animals. In all cases, data are presented as Mean ± SEM (Standard Error of Mean). The effect of the genotype on each subpopulation was assessed using ANOVA for repeated measurements and Student's T test. For quantification on embryonic sections, at least 3 pairs of littermates were used in each case (Rac1+/fl;Nkx2.1+/Cre;Rac3+/- vs Rac1fl/fl;Nkx2.1+/Cre;Rac3-/-). For each pair, four sections were used for confocal microscopy, all containing the region corresponding to the MGE, from rostral to caudal levels. From these four sections, an average number of cells were calculated for comparison of the two genotypes. The effect was assessed using ANOVA for repeated measurements, and Student's T test, and all data are presented as Mean ± SEM.

Results

Ablation of Rac1 and Rac3 from GABAergic interneurons leads to a delayed migration towards the developing neocortex.

The intracellular components that are coordinated the interneuron development are not fully understood. The Rac proteins a subfamily of RhoGTPases were found to be the crucial players in many processes as cytoskeleton organization, vesicle trafficking, transcription, cell cycle progression, and apoptosis (Jaffe & Hall, 2005).
In this study, we have exanimated the role of Racs in the early development of cortical GABAergic interneurons. Rac1 is ubiquitously expressed, while Rac3 is found exclusively in neurons. Rac1 KO is lethal, a conditional deletion of Rac1 exclusively only from the progenitors of cortical GABAergic interneurons is required. We conditionally ablated Rac1 from the place of origin of most cortical interneurons, the medial ganglionic eminence (MGE), by using the Nkx2.1-Cre line where specifically deletes Rac1 from the MGE (Vidaki et al., 2011). The pattern of expression of Nkx2.1-Cre transgene is very similar with the endogenous one (Fogarty et al., 2007). In addition to identify the cells where the ablation of Rac proteins occurs the reporter Rosa 26 Stop YFP which expresses (YFP) in a Cre-dependent manner was included (Srinivas et al., 2001).
Recent data from our team (Vidaki et al., 2011) characterizing this line points to a cell-autonomous and stage-specific requirement for Rac1 activity within proliferating interneurons for the co-ordination of cell cycle progression with differentiation and migration. As a result, 50% of GABAergic interneurons are absent from the postnatal cortex.
To study the role of both Rac proteins and the possibility that they could have synergistic effects the Rac1 conditional knockout was crossed with Rac3 KO as result we obtained a mouse line were interneurons deriving from MGE are missing the two Rac proteins Rac1 and Rac3. Rac1 conditional knock out mice die after 4 weeks, in contrast to Rac1 and Rac3 deficient mice die after postnatal day 15 (P15).

Work in our lab mainly performed by Dr Simona Tivodar showed that there are early defects in the migration of GABAergic interneruons, starting at E12.5. *Furthermore, analysis at E13.5 through E16.5 showed* that ablation of Rac1 and Rac3 from cortical interneuron progenitors results in an important delay of migration of these neurons towards the developing neocortex. These experiments revealed the position of interneurons marked with YFP via immunohistochemistry on coronal forebrain sections.

The number of MGE-derived interneuron subpopulations is severely reduced in the Rac1/Rac3 mutant postnatal brains.

At birth the double mutant pups look smaller than their control littermates and are alive only for two weeks. By using coronal sections from P5 brains with Nissl staining we analyzed the morphology but no gross defects were observed (***Figure 1***). To check the cortical layer formation in detail the situ hybridization technique was used in order to delimitate different cortical layers. Using specific markers as Rac3, Ror beta, ER81 and Cux2 we delimitated that all the layers are present, at the correct place where they have to be, but the cortex where both Rac proteins are missing is smaller (***Figure 1***).
To understand what happens postnatally with MGE-derived cells we analyzed first the YFP distribution in the cortex. We observed an important reduction of YFP-positive cells. Indeed, after quantification of YFP-positive cells at the level of barrel cortex we observed that in the double mutant almost 80% are absent when compared with the number from the control animals (*Figure 2A*). Then we wanted to characterize different subtypes of cortical interneurons that originated from MGE. To find out if different subtypes of interneurons are affected, double immunostaining was performed using different specific markers for MGE-derived interneurons: GABA, Lhx6, PVA, Som (***Figure 3***). Lhx6 is a post mitotic marker that is expressed by all the MGE-derived cells. After quantification of double positive Lhx6 and YFP only about 20% of these interneurons are found to be present in double mutant P5 cortex (***Figure 2B***). GABA is general marker of GABAergic interneurons. The quantification of the double positive GABA/YFP cells showed that also these double positive cells are missed in the same proportion as Lhx6/YFP in the Rac1 and Rac3 mutant cortex (***Figure 2C***). To further characterize the effect of the absence of Rac1 and Rac3 proteins on different interneuron subtypes we analyze the distribution of PVA/YFP (***Figure 2D***) and somatostatin positive cells (***Figure 2E***). As the histograms indicate, the same big reduction (almost 80%) was found also for these subtypes of cortical interneurons.
The major source of cortical interneurons is MGE where almost 70% of these cells originate, the second one being the CGE (Lee et al., 2010, Rudy et al., 2011). Most of the CR, VIP and NPY interneurons originate in the CGE. We analyzed as well the possibility that also these cells could increase in number in order to compensate for the high reduction of MGE-derived interneurons (***Figure 4***). The quantification of these interneuron subtypes demonstrated that the number of CGE-derived cells is not altered in the mutant cortex (***Figure 2F* and data not shown**). The percentage of double positive Lhx6;YFP, GABA;YFP and PVA;YFP over the total number of YFP positive cells demonstrated that this ratio is slightly diminished in the Rac1/3 double mutants. This data revealed that the defect is not only a migration defect but possibly also a differentiation defect during the development of the MGE-derived cells that are missing the two Rac proteins. However this effect is not as significant as the migration one.

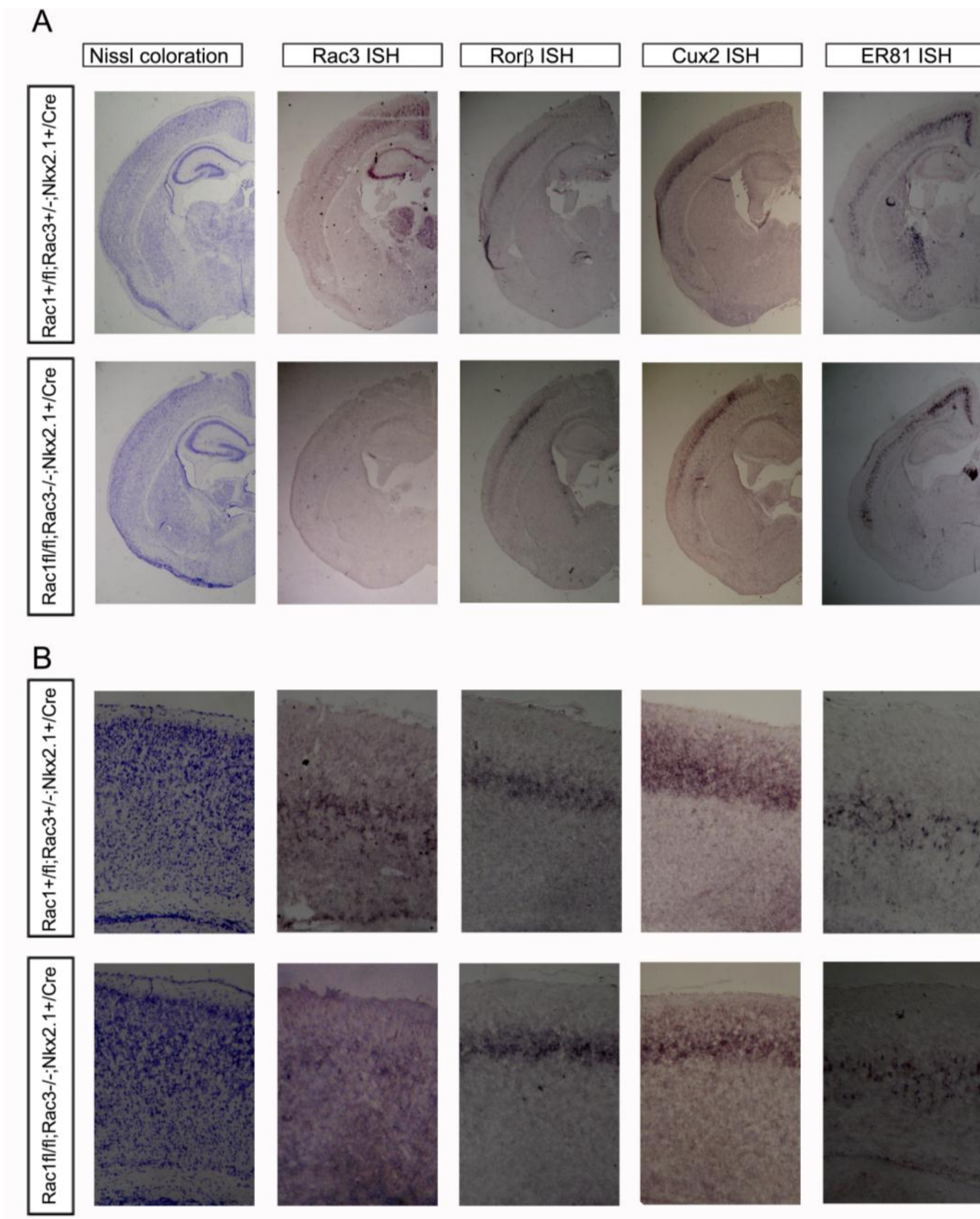

Figure 1. **Cortical lamination is not affected by the deletion of Rac1 and Rac3.** Coronal sections from P5 brains were analyzed by Nissl coloration and in situ hybridization using different markers for cortical layers Rac3 for layer IV, Ror beta for layer IV, Cux2 for upper layers and ER81 for layer V. (**B**) High magnification representation of the barrel cortex area.

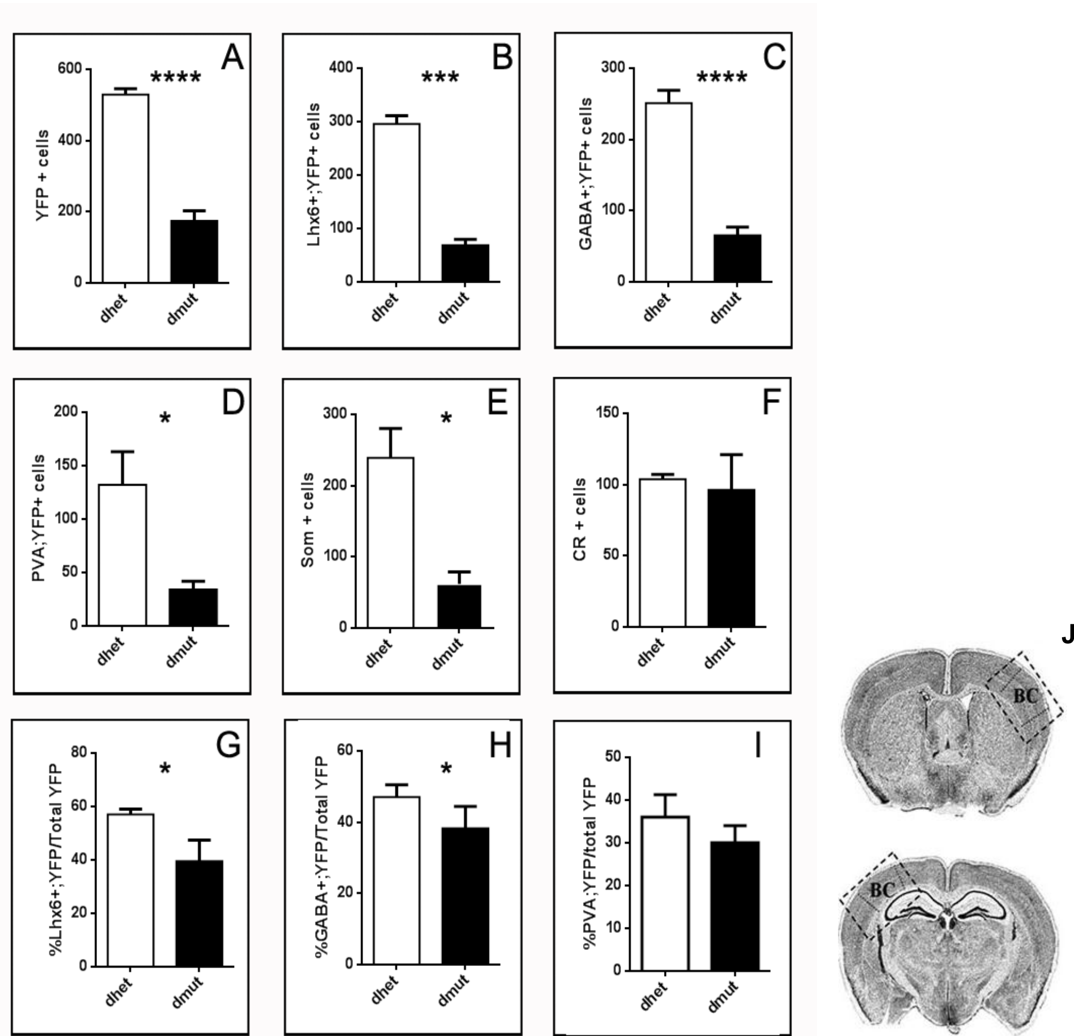

Figure 2. **Distribution of different cortical interneuron subtypes in the postnatal barrel cortex.** Distribution of different interneurons subtypes in P5 (A-C, E-H) and P15 (D, I) brains was analyzed using specific markers of GABAergic interneurons. The number of YFP+ (A), Lhx6+;YFP+ (B), GABA+;YFP+ (C), PVA+;YFP+ (D) and somatostatin+ (E) interneurons is reduced to almost 80% in the double mutants compared with the control animals. The number of CR positive cells is not affected in the absence of Rac1/3 proteins, the major part of these interneurons derive from CGE (F). The percentage of double positive Lhx6;YFP, GABA;YFP and PVA;YFP over the total number of YFP positive cells is different between control and mutant barrel cortex (G-I). (J) Representation of the brain sections that were used for counting. The dashed boxes indicate the area counted on each bregma, which includes the barrel cortex field (BC) between the lines. Statistical significance was assessed, using ANOVA for repeated measurements and Student's T test (P value <<0,05). Dr Tivodar has performed the quantifications for the P5 animals while I performed the ones at P15.

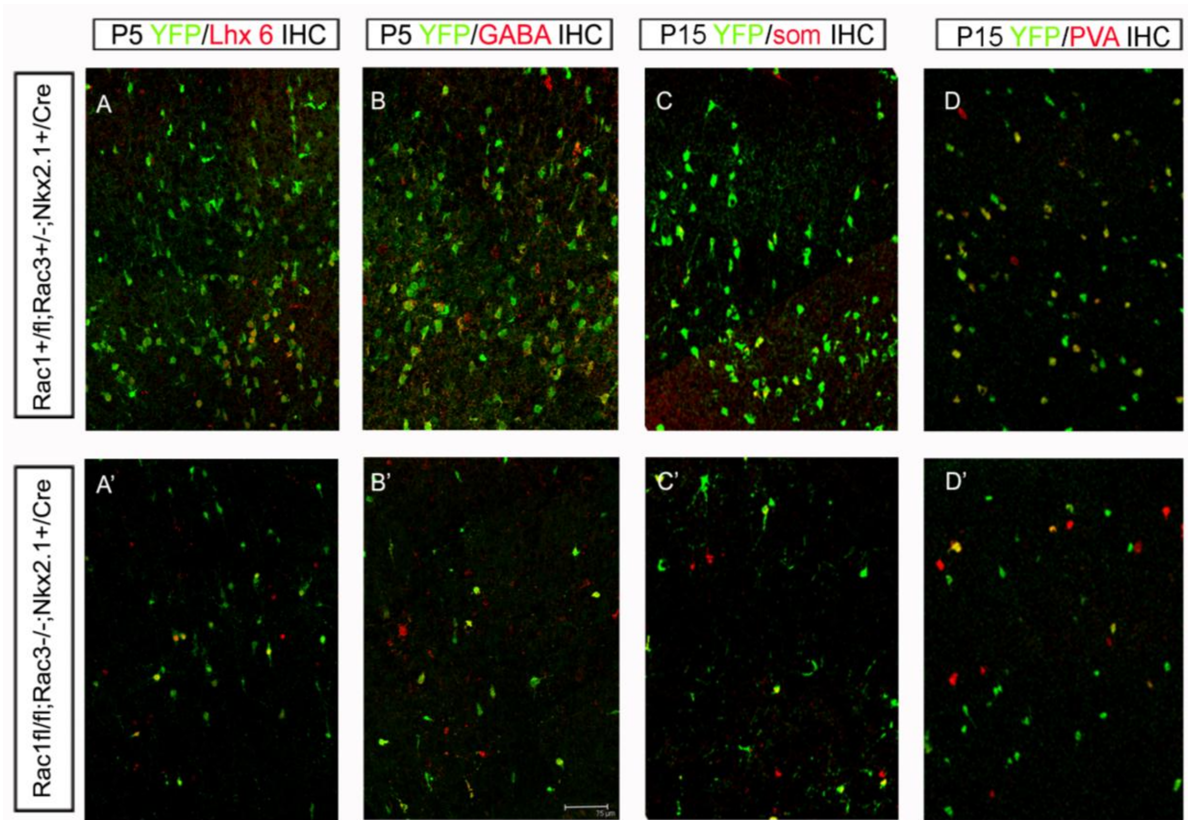

Figure 3. **Alteration of the distribution of MGE-derived interneurons in the absence of Rac1 and Rac3 proteins.** Coronal sections from P5 and P15 brains are analyzed for specific markers of GABAergic interneurons: **Lhx6**/YFP (A,A'), **GABA**/YFP (B,B'), Som (C,C'), **PVA**/YFP (D,D'). Scale bars: 25µm.

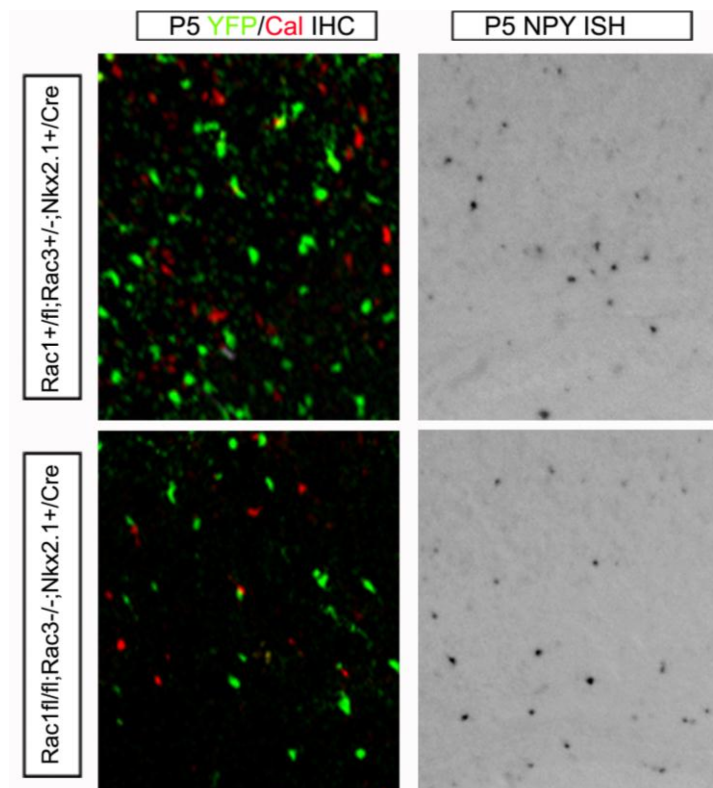

Figure 4. **Ablation of Rac1 and Rac3 proteins do not affect the CGE- derived interneurons.** Coronal sections from P5 brains are analyzed for specific markers of GABAergic interneurons derived from CGE: Carletinin (Cal) and NPY. Scale bars: 25µm.

The number of PVA$^+$/YFP$^-$ interneuron subpopulations is increased in the Rac1/Rac3 mutant brains.

Analysis ofP15 brains revealed that the number of PVA$^+$/YFP$^-$ interneurons is increased in Rac1 and Rac3-deficient mice compared to controls (***Figure 3 D,D' and Figure 5A***) meaning that either these cells are YFP-negative or in these cells the Cre recombinase is not active. These results raise a lot questions about the origin of these cells. Based on recentl studies the MGE gives rise to most PV-containing and SST-containing interneurons (Gelman et al., 2012). As a consequence, these cells, which are PVA-positive but not YFP-positive, may have a different origin other than the MGE. For this reason we used VIP to double-stain these cells, and our analysis of this immunostaining showed that PVA-positive cells are not overlapping with VIP-positive cells (data not show). Interestingly, all PVA$^+$/YFP$^-$ are Lhx6 positive (***Figure 5B***). Taken together, the data above showed that the P15 Rac1 and Rac3 deficient cortex is characterized by a reduced number of PVA$^+$/YFP$^+$ (*Figure 2*) and increased number of PVA$^+$/YFP$^-$ interneurons which are Lhx6 positive. Lhx6 is a post mitotic marker that is expressed by all the MGE derived cells (*Liodis et al., 2007*). Also, the fate of the large majority of PV- and SST- interneurons depends on *Lhx6*, a direct target of Nkx2-1 (*Du et al., 2008*).

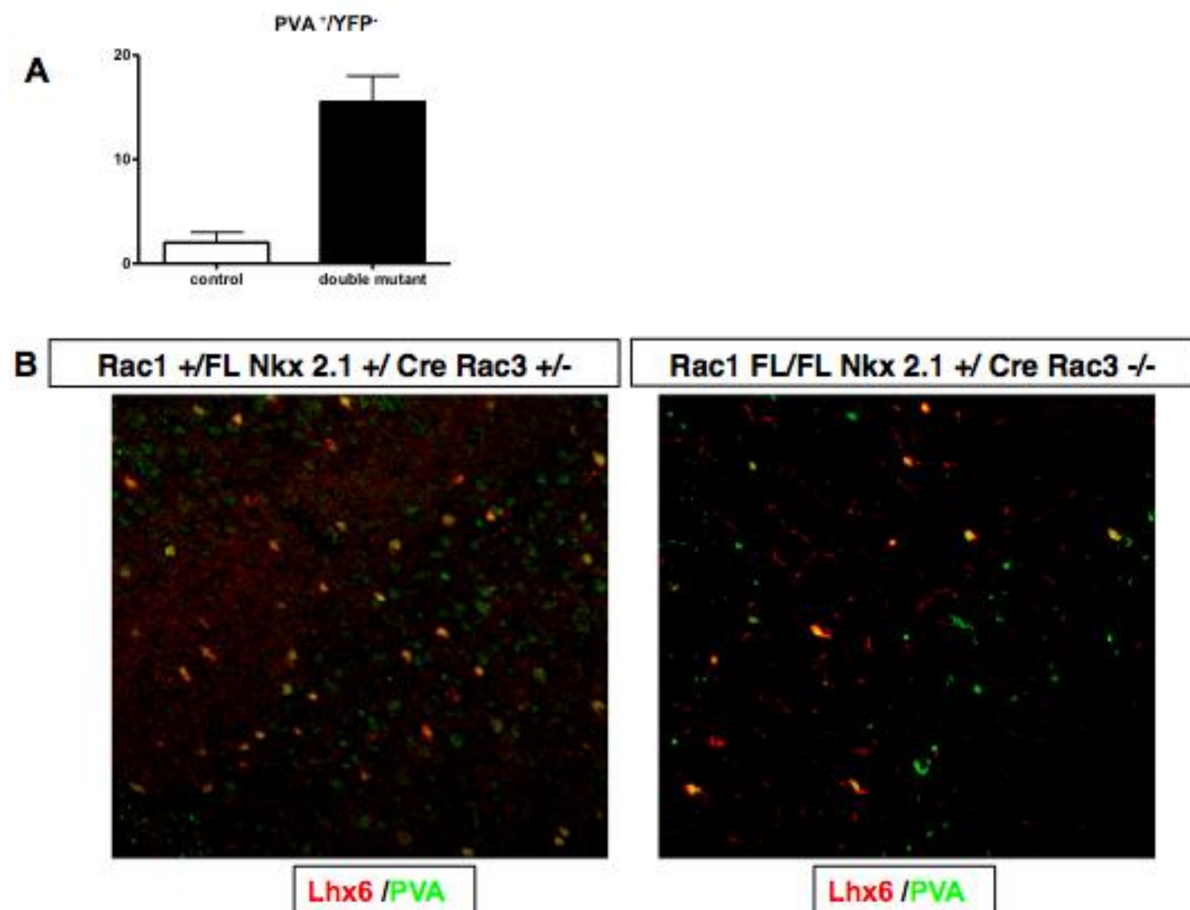

Figure 5. **The number of PVA+/YFP- interneuron subpopulations is increased in the Rac1/Rac3 mutant brains.** (A) The number of PVA$^+$/YFP$^-$ is increased in double mutant compare to control. Statistical significance was assessed, using ANOVA for repeated measurements and Student's T test

(P value <<0,05). (B) Coronal sections from P15 brains are analyzed for specific markers of GABAergic interneurons markers: PVA (green) and Lhx6 (red). All PVA^+ are $Lxh6^+$. Scale bars: 25μm.

Discussion

In this study, we show that the specific ablation of Rac1 function from Nkx2.1-expressing MGE-derived progenitors in Rac3 knock out mice leads to decrease migration of cortical interneurons from the MGE toward the developing cortex, which results in a severely reduced number of all MGE-derived neurons in the adult brain.

At early embryonic stages, ablation of Rac1 and Rac3 from cortical interneuron progenitors determines an important delay of migration of these neurons towards the developing neocortex. In other words Rac1 and Rac3 deficient interneurons fail to migrate toward the developing neorcortex, compared with the control cells and it is confirmed by specific markers of GABAergic interneurons. Later on, at E16.5 some of the Rac1 and Rac3 deficient cells manage to reach the cortex, although most of the cells remain aggregated at the ventral telencephalon. As a result, significantly reduced $GABA^+/YFP^+$ interneurons are present in the P5 and P15 mutant brains compared with the control (***Figure 2***). At these stages, we observed that double mutant brains are smaller than control. At P5, all layers are present, at the correct place where they have to be, but the cortex where both Rac proteins are missing is smaller. At P15 the size of cortex of Rac1 and Rac3 deficient mice is more orless the same as control mice and all layers are present, at the correct place where they have to be (*data not shown*).

A recent study from our lab using the Rac1 conditional knock out revealed that the specific ablation of Rac1 function from Nkx2.1-expressing MGE-derived progenitors leads to a perturbation of their cell cycle exit accompanied by decreased levels of cyclin D proteins and reduced phosphorylation of Rb, a critical checkpoint of the cell cycle. These results suggest that cells do not progress normally through cell cycle and a large number of them fail to migrate out of the MGE, thus aggregating in the ventral telencephalon. As a consequence, we observe decreased migration of cortical interneurons from the MGE toward the developing cortex, which results in a severely reduced number of all MGE-derived neurons in the adult brain (50% reduction of GABAergic interneurons from the postnatal cortex.). In contrast, ablation of Rac1 from Lhx6-positive interneurons that have already become postmitotic does not affect their development, further confirming the necessity of Rac1 in Nkx2.1-positive progenitors in order for them to complete their cell cycle (Vidaki et al., 2011). The majority of these mice die after 4 weeks of age due to epileptic seizures because 50% of GABAergic interneurons are absent from the postnatal cortex.

With this study we show that the absence of Rac3 in the Rac1-deficient interneurons has an additive effect. Only 20% of cortical interneurons are present in the cortex of mice missing Rac1 and 3 and the mice die even earlier than the Rac1 single mutants. **The progenitors of these cells also show a delay in cell cycle exit** (*data not shown*). In addition, MGE cells in vitro show cytoskeletal alterations such as a significant reduction of the leading process length and in growth cone formation in the absence of Rac1 protein, while Rac1/Rac3-deficient MGE cells show different, even more pronounced cytoskeletal defects. The absence of Rac1/3 could affect the stabilization of microtubules resulting in improper formation of leading processes. Indeed, the leading processes of Rac1/3-deficient MGE-derived cells in culture are severely impaired (*data not shown*). These data are consistent with previously

reported roles of Rac1 in cytoskeletal dynamics (Jaffe and Hall 2005; Watabe-Uchida et al. 2006; Koh 2007; Tahirovic et al. 2010).

The number of MGE-derived interneuron subtypes is highly reduced in the adult Rac1 and Rac3 mutant brains. At P15 Rac1 and Rac3deficient brains, the number of YFP positive interneurons is reduced to 80% compared with the control. In agreement with this, Lhx6 positive cells are also severely reduced in the double mutants and so are most of the subpopulations that derive from Lhx6 precursors, like PV and somatostatin (***Figure 2***). The percentage of double positive Lhx6;YFP, GABA;YFP and PVA;YFP over the total number of YFP-positive cells demonstrated that this ratio is diminished in the Rac1/3 double mutants, although not very significantly. This data revealed that the defect is not only a migration defect but a differentiation defect that may occur during the development of the MGE-derived cells missing the two Rac proteins. However, in the Rac1-deficient brains, the percentage of Lhx6;YFP, PV;YFP and Som;YFP double-positive cells over the total number of YFP cells in the cortex remains unaffected, indicating a migration rather than a differentiation defect of these cells (Vidaki et al., 2011). The number of calretinin and NPY positive interneurons is not altered in the Rac1 and Rac3 deficient brains (***Figure 2, 4***). These subpopulations are mainly derived from the CGE, where Nkx2.1 is not expressed.

In addition, at P15, Rac1 and Rac3-deficient cortex exhibits a reduced number of PVA^+/YFP^+ (*Figure 2*) and increased number of PVA^+/YFP^- interneurons which are Lhx6 positive. Lhx6 is a post mitotic marker that is expressed by all the MGE derived cells (Liodis et al., 2007). Also, the fate of the large majority of PV- and SST- interneurons depends on *Lhx6*, a direct target of Nkx2-1 (Du et al., 2008). Taken together, we observed that PVA/Lhx6-positive cells were YFP negative, in Rac1 and Rac3-deficient cortex, at P15 raising questions about their origin or mode of differentiation. According to the pattern of expression of Nkx2.1-Cre (which we have used for our animals) when crossed to the Cre reporter mouse R26R–GFP, there is a near complete recombination and activation of YFP in the neuroepithelium of the central and ventral MGE and the anterior endopenducular area (AEP) (Fogarty et al., 2007). As result, Rac1 is conditionally ablated by central and ventral MGE and the anterior endopenducular area (AEP) but not from dorsal MGE in double mutant. Overall, a possible explanation, about the origin of PVA-positive cells which are Lhx6-positive but not Nkx2.1-positive, is that these cells derived from dorsal MGE. This hypothesis has to be tested. Subsequent studies have focused on the functions of Nkx2-1 and other transcription factors in the development of distinct interneuron subtypes. For example, acquisition of the fast-spiking characteristics and expression of PV is regulated by the actions of the Nkx2-1, Dlx5, Dlx6, Lhx6 and Sox6 transcription factors (Liodis et al., 2007; Butt, et al., 2008; Wang et al., 2010; Azim et al., 2009). Consequently, it will be interesting to check if these cells have the morphological and electrophysiological properties characteristic of PV-positive interneurons.

In summary, our genetic models combined with the severe reduction of cortical interneurons observed, are indispensable tools in deciphering the intracellular mediators of interneuron development. As far as we know, these are the only genetically modified mice where ablation of an intracellular modulator results in such a severe reduction in interneurons.

Reference

Ammer AG, Weed SA. (2008). Cortactin branches out: roles in regulating protrusive actin dynamics. Cell Motil Cytoskeleton. 65:687--707.

Anderson, S. A., Marin, O., Horn, C., Jennings, K. & Rubenstein, J. L. (2001) Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128, 353–363

Azim E, Jabaudon D, Fame RM, Macklis JD. SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. Nat Neurosci 2009;12:1238–1247

Barnes A. P, Solecki D., Polleux F. (2008) New insights into the molecular mechanisms specifying neuronal polarity in vivo Current Opinion in Neurobiology 18(1):44-52.

Butt SJ, Sousa VH, Fuccillo MV, Hjerling-Leffler J, Miyoshi G, Kimura S, Fishell G. (2008). The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron. 59:722--732.

Chen, L., Melendez, J., Campbell, K., Kuan, C. Y., and Zheng, Y. (2009). Rac1 deficiency in the forebrain results in neural progenitor reduction and microcephaly. Dev Biol 325, 162-170.

Chen, L., Liao, G., Waclaw, R. R., Burns, K. A., Linquist, D., Campbell, K., Zheng, Y., and Kuan, C. Y. (2007). Rac1 controls the formation of midline commissures and the competency of tangential migration in ventral telencephalic neurons. J Neurosci 27, 3884-3893.

Chen Z, Sun J, Pradines A, Favre G, Adnane J, Sebti SM (2000) Both farnesylated and geranylgeranylated RhoB inhibit malignant transformation and suppress human tumor growth in nude mice. J Biol Chem 275(24):17974–17978

Cobos, I., Calcagnotto, M. E., Vilaythong, A. J., Thwin, M. T., Noebels, J. L., Baraban, S. C., and Rubenstein, J. L. (2005). Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy [see comment]. Nat. Neurosci. 8, 1059–1068.

Colicelli J. 2004. Human RAS superfamily proteins and related GTPases. Sci STKE 2004: RE13.

Corbetta, S., Gualdoni, S., Albertinazzi, C., Paris, S., Croci, L., Consalez, G. G., and de Curtis, I. (2005) Generation and characterization of Rac3 knockout mice. Mol. Cell. Biol. 25, 5763–5776

Corbin, J.G., Nery, S., Fishell, G., (2001). Telencephalic cells take a tangent: nonradial migration in the mammalian forebrain. Nat. Neurosci. 4 (Suppl. 1), 1177–1182.

Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R. & Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett Syndrome. Proc. Natl Acad. Sci. USA, 102, 12560–12565.

Denaxa M, Chan CH, Schachner M, Parnavelas JG, Karagogeos D. 2001. The adhesion molecule TAG-1 mediates the migration of cortical interneurons from the ganglionic eminence along the corticofugal fiber system. Development. 128:4635--4644.

Didsbury JR, Uhing RJ, Snyderman R (1990) Isoprenylation of the low molecular mass GTP-binding proteins Rac1 and Rac2: possible role in membrane localization. Biochem Biophys Res Commun 171:804–812

Du, T., Xu, Q., Ocbina, P. J., and Anderson, S. A. (2008). NKX2.1 specifies cortical interneuron fate by activating Lhx6. Development 135, 1559–1567.

Etienne-Manneville S, Hall A (2002) Rho GTPases in cell biology. Nature 420:629–635

Faux C, Rakic S, Andrews W, Yanagawa Y, Obata K, Parnavelas JG. (2010). Differential gene expression in migrating cortical interneurons during mouse forebrain development. J Comp Neurol. 518(8):1232-48.

Fazzari, P., Paternain, A.V., Valiente, M., Pla, R., Lujan, R., Lloyd, K., Lerma, J., Marin, O. & Rico, B. (2010) Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. Nature, 464, 1376–1380.

Flames, N., Pla, R., Gelman, D.M., Rubenstein, J.L.R., Puelles, L. & Marin, O. (2007) Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J. Neurosci., 27, 9682– 9695.

Fogarty, M., Grist, M., Gelman, D., Marin, O., Pachnis, V., and Kessaris, N. (2007). Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. J. Neurosci. 27, 10935–10946.

Frost, J.A., Swantek, J.L., Stippec, S., Yin, M.J., Gaynor, R., Cobb, M.H. (2000)..Stimulation of NFκB Activity by Multiple Signaling Pathways Requires PAK1. J. Biol. Chem. 275, 19693–19699

Fukata Y, Kimura T, Kaibuchi K. (2002). Axon specification in hippocampal neurons. Neurosci Res. 43:305—315.

Fuchs, S., Herzog, D., Sumara, G., Buchmann-Moller, S., Civenni, G., Wu, X., Chrostek-Grashoff, A., Suter, U., Ricci, R., Relvas, J. B., et al. (2009). Stage-specific control of neural crest stem cell proliferation by the small rho GTPases Cdc42 and Rac1. Cell Stem Cell 4, 236-247.

Gelman, D.M., Martini, F.J., Nobrega-Pereira, S., Pierani, A., Kessaris, N. & Marin, O. (2009). The embryonic preoptic area is a novel source of cortical GABAergic interneurons. J. Neurosci., 29, 9380–9389.

Gelman DM, Marín; O, Rubenstein JLR. (2012). The Generation of Cortical Interneurons. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US).

Gelman DM, Marın O. Generation of interneuron diversity in the mouse cerebral cortex (2010). Eur J Neurosci 31(12):2136–2141.

Gardiner EM, Pestonjamasp KN, Bohl BP, Chamberlain C, Hahn KM, Bokoch GM. 2002. Spatial and temporal analysis of Rac activation during live neutrophil chemotaxis. Curr Biol. 12:2029--2034.

Grigoriou, M., Tucker, A. S., Sharpe, P. T., and Pachnis, V. (1998). Expression and regulation of Lhx6 and Lhx7, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development. Development 125, 2063–2074.

Goode BL, Eck MJ. (2007) Mechanism and Function of Formins in the Control of Actin Assembly. Annu Rev Biochem. 76:593-627.

Heck N., Kilb W., Reiprich P., Kubota H., Furukawa T., Fukuda A. and LuhmannH. (2007) GABA-A Receptors Regulate Neocortical Neuronal Migration In Vitro and In Vivo. Cerebral Cortex 17:138--148

Heng JI, et al. (2008) Neurogenin 2 controls cortical neuron migration through regulation of Rnd2. Nature 455:114–118.

Itoh RE, Kurokawa K, Ohba Y, Yoshizaki H, Mochizuki N, Matsuda M. (2002). Activation of rac and cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. Mol Cell Biol. 22:6582--6591.

Jacobs T, Causeret F, Nishimura YV, Terao M, Norman A, Hoshino M, Nikolić M. (2007) Localized activation of p21-activated kinase controls neuronal polarity and morphology. J Neurosci. 27(32):8604-15.

Jaffe AB, Hall A. (2005). Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol. 21:247--269.

Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D'Amico, M., Steer,J., Klein, J.U., Lee, R.J., Segall, J.E., Westwick, J.K., et al. (1999). Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway. J. Biol. Chem. 274, 25245–25249.

Kawauchi, T., Chihama, K., Nabeshima, Y., and Hoshino, M. (2003). The in vivo roles of STEF/Tiam1, Rac1 and JNK in cortical neuronal migration. Embo J 22, 4190-4201.

Koh CG. (2007). Rho GTPases and their regulators in neuronal functions and development. Neurosignals. 15:228--237.

Kriegstein, A.R., and Noctor, S.C. (2004). Patterns of neuronal migration in the embryonic cortex. Trends Neurosci. 27, 392–399.

Kyriakopoulou K, de Diego I, Wassef M, Karagogeos D. 2002. A combination of chain and neurophilic migration involving the adhesion molecule TAG-1 in the caudal medulla. Development. 129:287--296.

Lavdas, A. A., Grigoriou, M., Pachnis, V., and Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J. Neurosci. 19, 7881–7888.

Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. (2010). The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. J Neurosci, in press.

Levitt, P. (2005) Disruption of interneuron development. Epilepsia, 46, 22–28.

Lewis, D.A., Hashimoto, T. & Volk, D.W. (2005) Cortical inhibitory neurons and schizophrenia. Nat. Rev. Neurosci., 6, 312–324.

Liodis, P., Denaxa, M., Grigoriou, M., Akufo-Addo, C., Yanagawa, Y., and Pachnis, V.
(2007). Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. J. Neurosci. 27, 3078–3089.

Malosio ML, Gilardelli D, Paris S, Albertinazzi C, de Curtis I. (1997). Differential expression of distinct members of Rho family GTPbinding proteins during neuronal development: identification of Rac1B, a new neural-specific member of the family. J Neurosci. 890 17:6717--6728.

Marin, O., Rubenstein, J.L., (2001). A long, remarkable journey: tangential migration in the telencephalon. Nat. Rev., Neurosci. 2, 780–790.
Marin, O., and Rubenstein, J.L. (2003). Cell migration in the forebrain. Annu. Rev. Neurosci. 26, 441–483.

Martini FJ, Valiente M, Lopez Bendito G, Szabo G, Moya F, Valdeolmillos M, Marin O. (2009). Biased selection of leading process branches mediates chemotaxis during tangential neuronal migration. Development. 136:41--50.

Métin, C., Baudoin, J. P., Rakic´, S. and Parnavelas, J. G. (2006). Cell and molecular mechanisms involved in the migration of cortical interneurons. Eur. J. Neurosci. 23, 894-900

Miki H, Suetsugu S, Takenawa T (1998): WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. EMBO J; 17: 6932–6941.

Miki H, Yamaguchi H, Suetsugu S, Takenawa T (2000): IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. Nature; 408: 732–735.

Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V.H., Butt, S.J.B., Battiste, J., Johnson, J.E., Machold, R.P. & Fishell, G. (2010) Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. J. Neurosci., 30, 1582–1594.

Ng J, Luo L. 2004. Rho GTPases regulate axon growth through convergent and divergent signaling pathways. Neuron 44: 779–793.

Nikolic, M., Chou, M. M., Lu, W., Mayer, B. J., and Tsai, L. H. (1998). The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. Nature 395, 194-198.

Nishimura T, Yamaguchi T, Kato K, Yoshizawa M, Nabeshima Y, Ohno S, Hoshino M, Kaibuchi K. (2005) PAR-6-PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. Nat Cell Biol. 7(3):270-7

Olson, M. F., Ashworth, A., and Hall, A. (1995). An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. Science 269, 1270-1272.

Olsson, M., Bjorklund, A., and Campbell, K. (1998). Early specification of striatal projection neurons and interneuronal subtypes in the lateral and medial ganglionic eminence. Neuroscience 84, 867–876.

Owens, D. F. & Kriegstein, A. R. (2002). Is there more to GABA than synaptic inhibition? Nature Rev. Neurosci. 3, 715–727

Parent JM, (2007) Adult neurogenesis in the intact and epileptic dentate gyrus. Prog Brain Res 163:529-40

Petilla Interneuron Nomenclature Group, Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsaki, G., Cauli, B., Defelipe, J., Faire´n, A., Feldmeyer, D., Fishell, G., et al. (2008). Petilla terminology: Nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat. Rev. Neurosci. 9, 557–568.

Rallu, M., Machold, R., Gaiano, N., Corbin, J. G., McMahon, A. P., and Fishell, G. (2002b). Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and Hedgehog signaling. Development 129, 4963–4974.

Ridley A. (2006). Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. Trends Cell Biol 16 (10): 522–9.

Rossman, K. L., Der, C. J. & Sondek, J. (2005). GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol 6, 167-180

Rubenstein, J.L.R. & Merzenich, M.M. (2003) Model of autism: increased ratio of excitation ⁄ inhibition in key neural systems. Genes Brain Behav., 2, 255–
267.

Rudy B, Fishell G, Lee S, Hjerling-Leffler J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev Neurobiol. 71(1):45-61

Sarner, S., Kozma, R., Ahmed, S., and Lim, L. (2000). Phosphatidylinositol 3-kinase, Cdc42, and Rac1 act downstream of Ras in integrin-dependent neurite outgrowth in N1E-115 neuroblastoma cells. Mol Cell Biol 20, 158-172

Sideropoulou, K. Pissadaki E. K. , Poirazi P. (2006) Inside the brain of a neuron. EMBO Rep. Sep;7(9):886-92.

Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol. 1:4.

Stenman, J., Toresson, H., and Campbell, K. (2003a). Identification of two distinct progenitor populations in the lateral ganglionic eminence: Implications for striatal and olfactory bulb neurogenesis. J. Neurosci. 23, 167–174.

Sussel, L., Marin, O., Kimura, S., and 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, 3359–3370.

Schwamborn, J. C., Fiore, R., Bagnard, D., Kappler, J., Kaltschmidt, C., and Puschel, A. W. (2004). Semaphorin 3A stimulates neurite extension and regulates gene expression in PC12 cells. J Biol Chem 279, 30923-30926.

Tahirovic S, Hellal F, Neukirchen D, Hindges R, Garvalov BK, Flynn KC, Stradal TE, Chrostek-Grashoff A, Brakebusch C, Bradke F. (2010). Rac1 regulates neuronal polarization through the WAVE complex. J Neurosci. 30:6930--6943.

Vidaki M., Tivodar S., Doulgeraki K., Tybulewicz V., Kessaris N., Pachnis V. and Karagogeos D. (2011) Rac1 Dependent Cell Cycle Exit of MGE Precursors and GABAergic Interneuron Migration to the Cortex. Cerebral Cortex 10.1093

Walmsley MJ, Ooi SK, Reynolds LF, Smith SH, Ruf S, Mathiot A, Vanes L, Williams DA, Cancro MP, Tybulewicz VL. 2003. Critical roles for Rac1 and Rac2 GTPases in B cell development and signaling. Science. 302:459--462.

Wang Y, Dye CA, Sohal V, Long JE, Estrada RC, Roztocil T, Lufkin T, Deisseroth K, Baraban SC, Rubenstein JLR. Dlx5 and Dlx6 Regulate the Development of Parvalbumin-Expressing Cortical Interneurons. J Neurosci 2010;30:5334–5345

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. USA 101, 1368–1373

Watabe-Uchida M, Govek EE, Van Aelst L. (2006). Regulators of Rho GTPases in neuronal development. J Neurosci. 26:10633—10635.

Westwick JK, Lambert QT, Clark GJ, Symons M, Van Aelst L, et al. (1997) Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways. Mol. Cell Biol. 17:1324–35

Whittington, M. A. & Traub, R. D. (2003). Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. Trends. Neurosci. 26, 676–682

Wichterle, H., Turnbull, D. H., Nery, S., Fishell, G. & Alvarez-Buylla, A. (2001). In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128, 3759–3771

Wonders, C.P., Anderson, S.A., (2006). The origin and specification of cortical interneurons. Nat. Rev., Neurosci. 7, 687–696.

Wonders, C. P., Taylor, L.,Welagen, J., Mbata, I. C., Xiang, J. Z., and Anderson, S. A. (2008). A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. Dev. Bio. 314, 127–136.

Yamato, K., Yamamoto, M., Hirano, Y., and Tsuchida, N. (1995). A human temperature-sensitive p53 mutant p53Val-138: modulation of the cell cycle, viability and expression of p53-responsive genes. Oncogene 11, 1-6.

Yoshizawa, M., Kawauchi, T., Sone, M., Nishimura, Y. V., Terao, M., Chihama, K., Nabeshima, Y., and Hoshino, M. (2005). Involvement of a Rac activator,P-Rex1, in neurotrophin-derived signaling and neuronal migration. J Neurosci 25, 4406-4419.