The role of GlyRa2 in interneuronal development

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INTRODUCTION

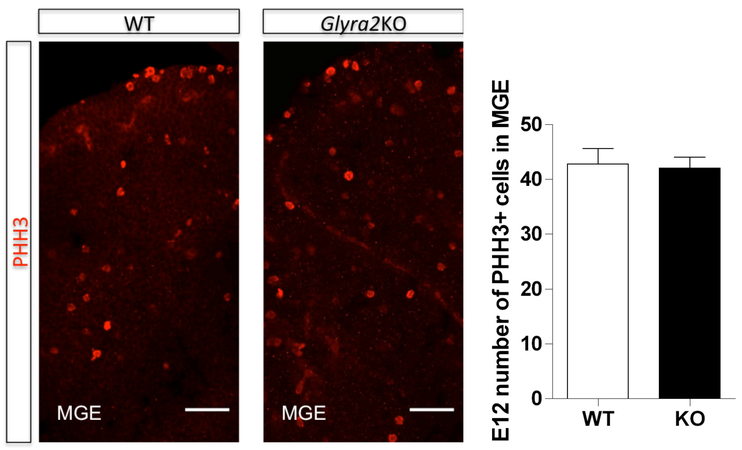
During development of the telencephalon, the medial ganglionic eminence (MGE) is the source of the majority of hippocampal interneurons and actual all of striatal interneurons. At E9 interneuronal proliferation of the MGE starts, reaching a peak at E13 (1). Subsequently, tangential migration will bring the divers pool of interneurons to their site of destination. The goal of this study is to observe and specify the role of the Glycine Receptor alpha 2 subunit (GlyRa2) in embryonic and early post-natal development with the focus on specific types of interneurons in striatum and hippocampus. Preliminary data have shown that *Glyra2* disruption causes a delay in interneuronal migration leading to a reduction in number of interneurons in the cortex at postnatal day zero (2,3). We hypothesized that the migratory defect and reduction in number of interneurons, previously observed, might be caused by a proliferative defect during early embryogenesis and cause a differential patterning of interneuronal populations which distribute differently in the brain. Here we report that there is no difference in mitotic rate between the two genotypes at E12. However, different immunohistochemical labelings demonstrated that there is a difference in the number of interneurons at P14 and P30.

MATERIALS AND METHODS

Immunohistochemistry: antibodies; Goat anti-ChAT (cholinergic interneuron marker) Rabbit anti-GFP (marker Dlx 5/6-GFP), Mouse anti-Parvalbumin, Rabbit anti-PHH3 (mitotic marker), Rat anti-Somatostatin
Imaging: fluorescent and confocal microscopy

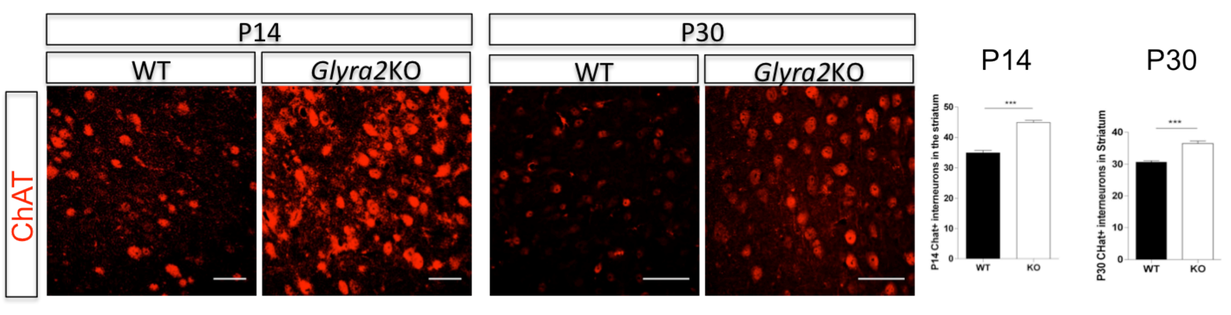
RESULTS

No alteration in proliferation of interneurons at E12 in the MGE



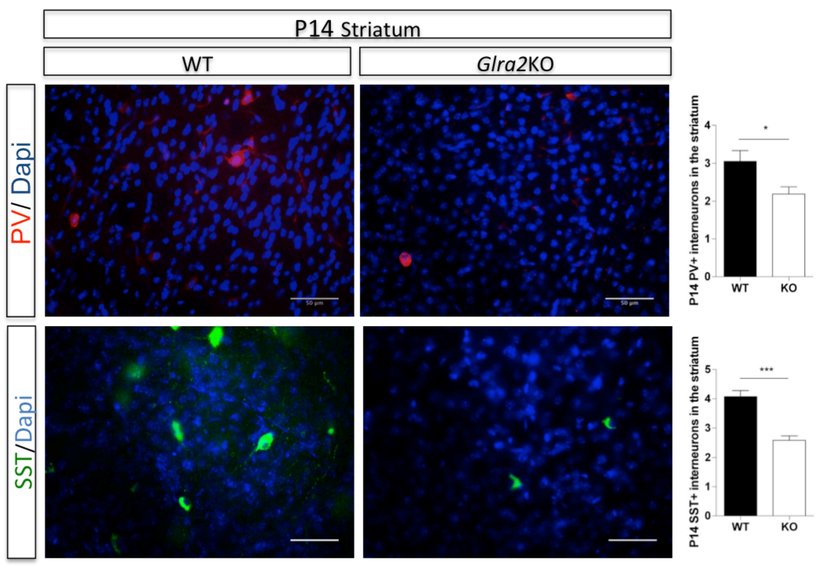
Immunolabeling of the PhosphoHiston-H3 (PHH3) positive cells (red) in the Medial Ganglionic eminence (MGE) at E12. PHH3 reflects a very small time window in cell cycle (M phase). PHH3 also exclude apoptotic cells and is thereby a very specific proliferation marker.
No difference observed among the genotypes. n= 3 animals for each condition. Scale bare: 50 µm.

Cholinergic interneuron cell population is increased in the striatum



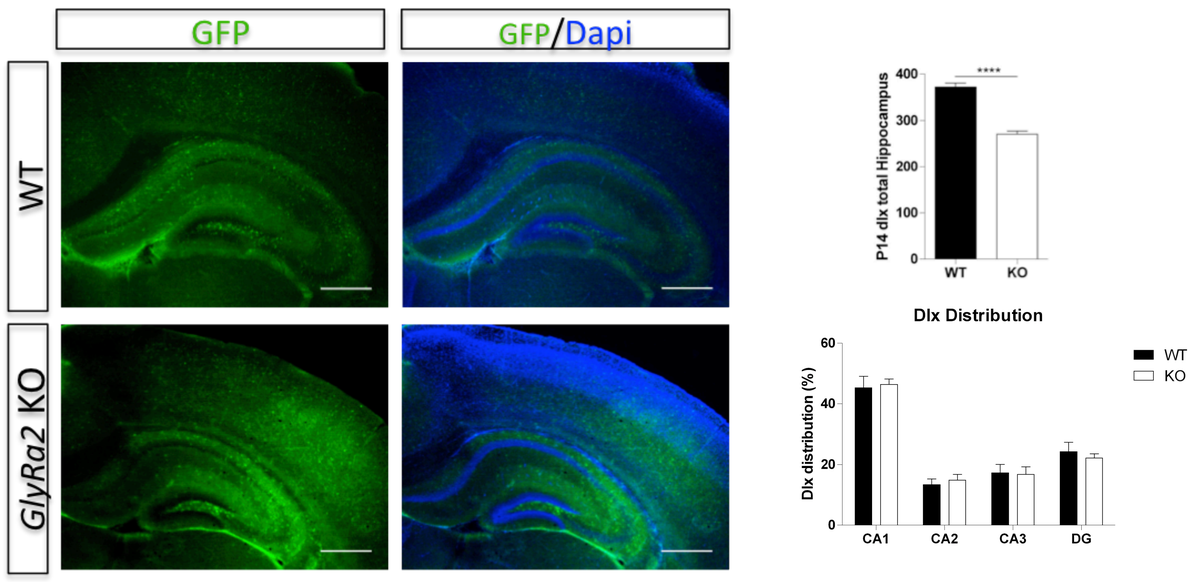
Immunolabeling for ChAT+ cholinergic interneurons (red) and Dapi (blue) at P14 and P30 showing an increase of interneurons in the striatum in *GlyRa2*KO mice. ***p< 0,001; n= 4 animals for each condition. Scale bare: 50 µm.

PV+ and SST+ interneuron are decreased in the striatum at P14



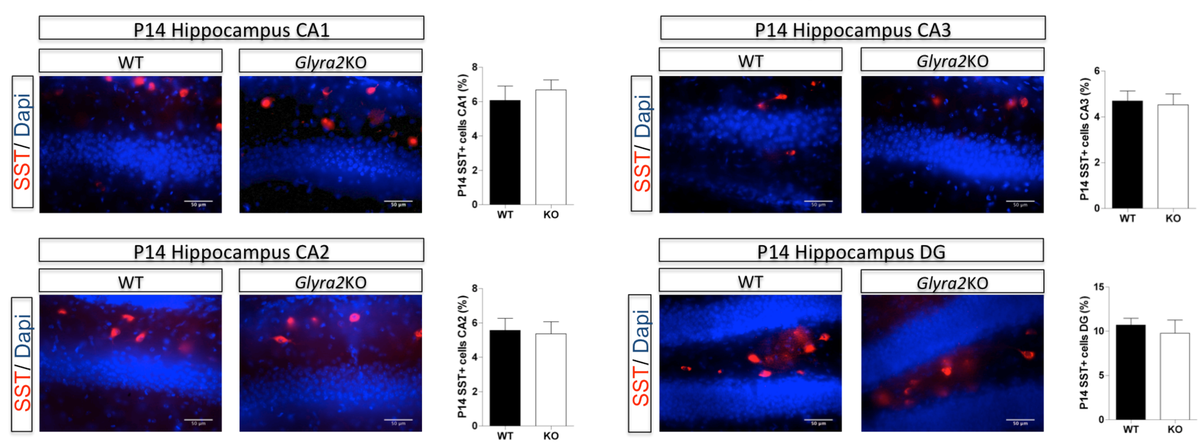
Immunolabeling for Parvalbumin (PV) positive interneurons (red, top panel), Somatostatin (SST) Positive (green, lower panel) and Dapi (blue) at P14 which illustrates an overall reduction of these GABAergic interneurons in the striatum in the *GlyRa2*KO mice. *p<0,05 ***p< 0,001; n= 4 animals for each condition. Scale bare: 50 µm.

A decrease of GABA-ergic interneurons in the hippocampus at P14



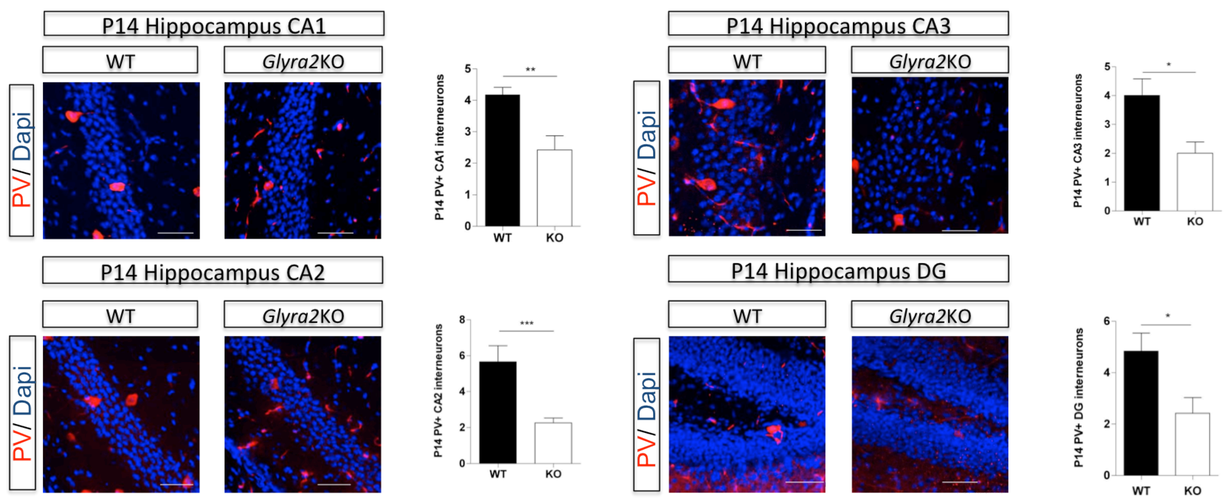
Immunolabeling of the hippocampal Dlx 5-6 GFP positive interneurons (green)and Dapi (blue) at P14 showing a decrease of interneurons in *GlyRa2*KO mice. The overall distribution in the divers hippocampal regions was not altered ****p< 0,0001; n= 3 animals for each condition. Scale bare: 50 µm.

The distribution of SST+ interneurons remains unchanged in the hippocampus at P14



Immunolabeling of the SST positive interneurons (red) and Dapi (blue) in the hippocampal regions at P14 displaying no significant difference in distribution between the two genotypes. The total number of SST positive hippocampal interneurons showed no difference between the genotypes (data not shown). n= 3 animals for each condition. Scale bare: 50 µm.

A decrease in PV+ interneuronal population in the hippocampal regions at P14



Immunolabeling for the PV positive interneurons (red) and Dapi (blue) in the different hippocampal regions at P14 displaying no significant difference in number between the two genotypes. *p<0,05 **p<0,01 ***p< 0,001; n= 3 animals for each condition. Scale bare: 50 µm.

CONCLUSION

Our results do not totally discard the two hypotheses:

1. The number cells in mitosis is not altered between the two genotypes.
2. *GlyRa2*KO mice display an increase of cholinergic interneurons in the striatum. However the number of GABAergic interneurons (SST and PV) are decreased
3. *GlyRa2*KO mice present a reduction in the amount of Dlx 5/6 positive interneurons in the hippocampus with a parallel reduction of PV^+ interneurons but no change in SST^+ interneurons.

To exclude a proliferation defect and to look for cell cycle defects and early differentiation, BrdU-experiments with co-labeling and at different time points should be performed. Either way our results already demonstrate that the GlyRa2 subunit has an important role in interneuronal development.

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