Reactive neural stem cells and aberrant neurogenesis in a neuron-specific model of Dravet Syndrome

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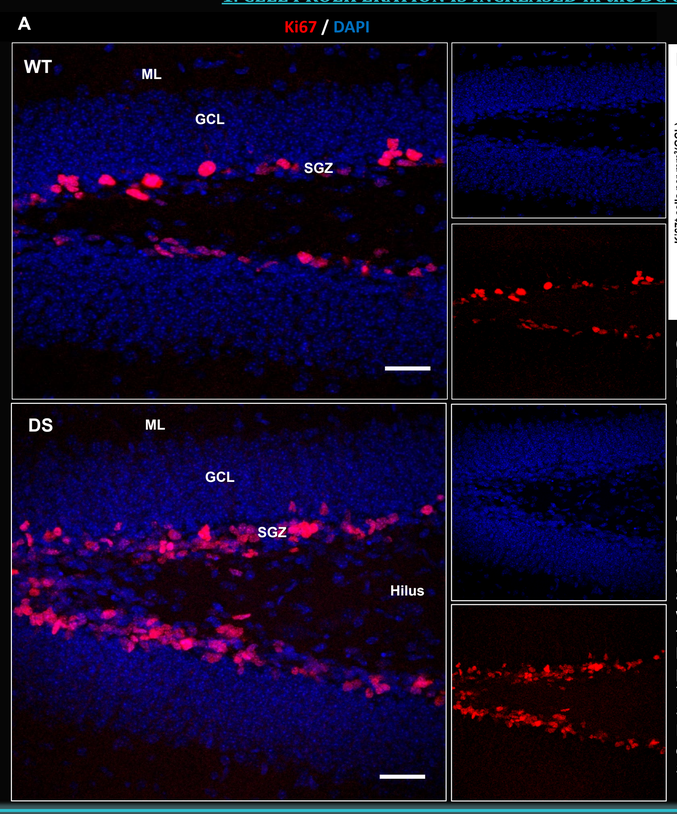
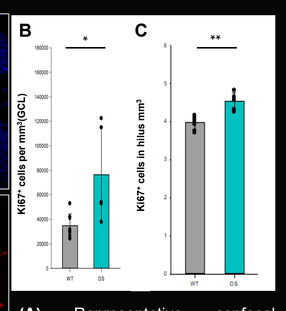
Hippocampal neurogenesis (HN) is a form of neuroplasticity which implicates the generation of new neurons from neural stem cells (NSCs) in the dentate gyrus (DG). Although HN persists throughout adulthood, it reaches maximum values during early postnatal periods, when the population of NSCs is at its largest. NSC activity and HN are particularly regulated by neuronal activity and severe alterations have been found in the hippocampal neurogenic niche in mouse models of epilepsy. Induction of reactive-like and gliogenic NSCs (React-NSCs) besides aberrant neurogenesis, defined by altered newborn neuron morphology, migration and functional properties, are induced by epileptic seizures. We are thus interested in Dravet Syndrome (DS), a severe form of infant epilepsy characterized by the early onset (3-6 months of age) of seizures. Hippocampus is the special interest in epilepsy, so as in DS, because it is related to higher cognitive functions involved in memory processes, learning and emotional regulation. DS is caused by mutations in the *Scn1a* gene encoding the α1 subunit of sodium channel Nav1.1, and provokes febrile seizures, hyperexcitability, neurological comorbidities and premature death. Therefore, we hypothesize that early seizures could have a greater impact and longer-lasting on the neurogenic niche in DS due to their early onset. Through confocal microscopy imaging we are analysing the neurogenic niche of a novel inducible knock-in mouse model of DS (Syn-Cre/Scn1a$^{WT/A1783V}$) at postnatal day 25 (soon after the onset of seizures) which consist in the neuron-targeted expression of a missense mutation (A1783V) in the *Scn1a* gene. We have observed the induction of React-NSCs, characterized by more and thickened branches plus overproliferation. We have also observed a strong induction of aberrant neurogenesis. Newborn immature neurons, identified by the expression of doublecortin are present in much higher numbers; migrate abnormally towards the hilus and the molecular layer; and have basal dendrites and V-shaped proximal apical dendrites. We are currently investigating other possible alterations such as cell death/survival, differentiation imbalance and changes in astroglia and microglia.

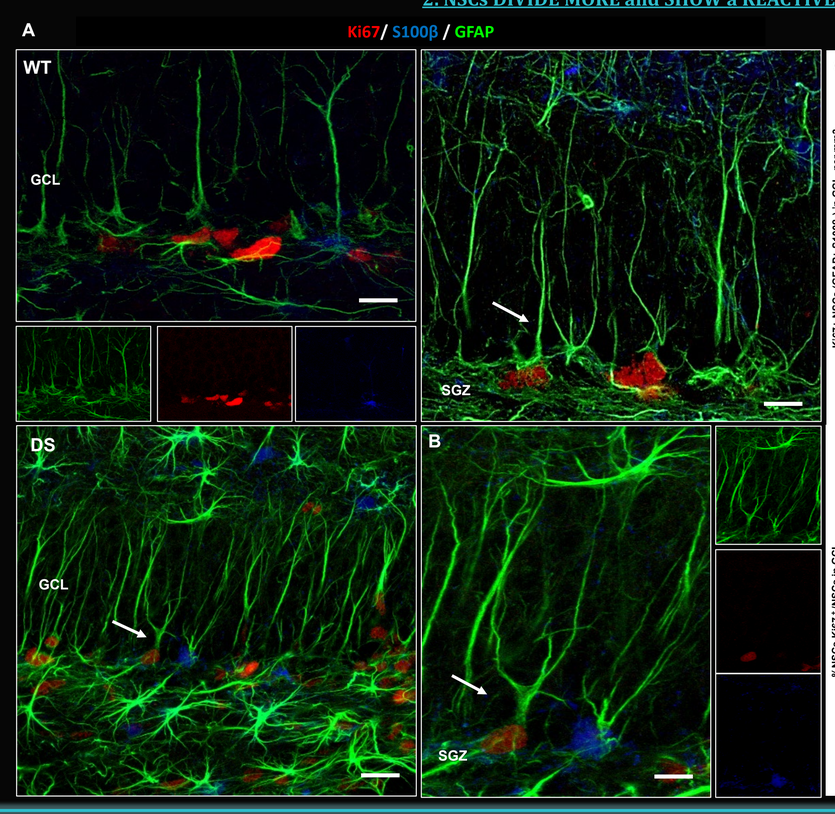
1. CELL PROLIFERATION IS INCREASED in the DG of DS MICE





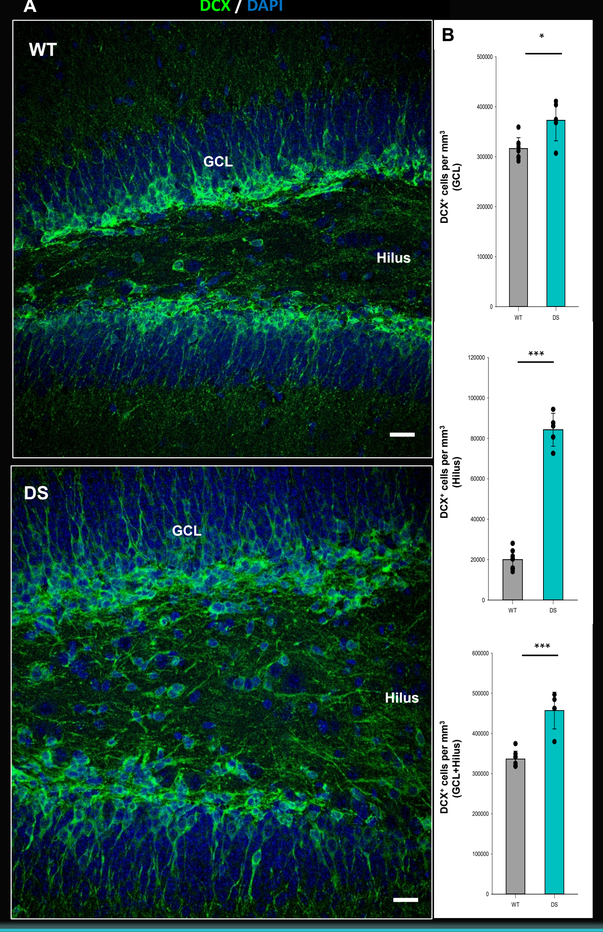
(A) Representative confocal-microscopy images of Ki67 immunostaining (red) and DAPI (blue) in 25-day old WT (up) and DS (down) mice. Cell proliferation is mostly restricted to the neurogenic niche (SGZ and proximal part of the hilus).
Cell proliferation, measured as density of Ki67+ cells, is significantly increased in the SGZ+GCL **(B)** and in the hilus **(C)** of DS mice. n = 8 for WT and n = 5 for DS. Data were analysed by Student's t-test, data were subjected to log10-simple transformation when homocedasticity test was not passed. *p= *p < 0.05, **p < 0.01, ***p < 0.001. Bars show mean ± SEM. Dots show individual data. ML, molecular layer. GCL, granule cell layer. SGZ, subgranular zone. Scale bar is 20 µm.

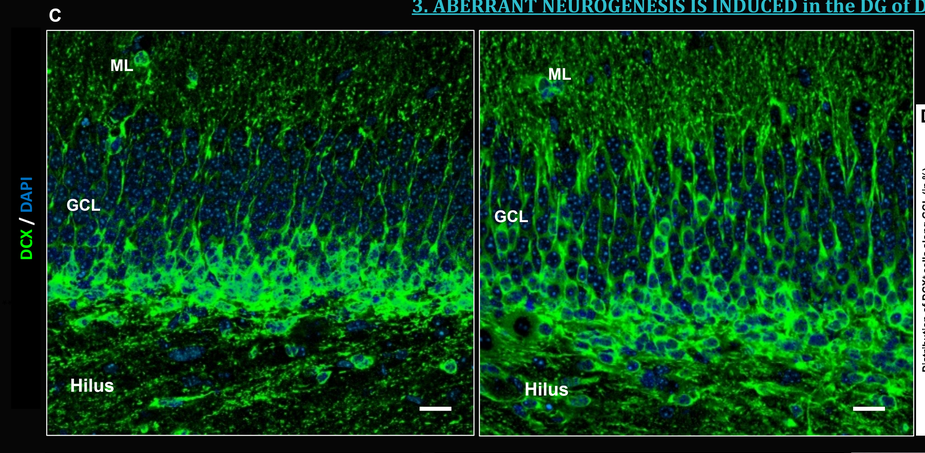
2. NSCs DIVIDE MORE and SHOW a REACTIVE PHENOTYPE in the DG of DS mice

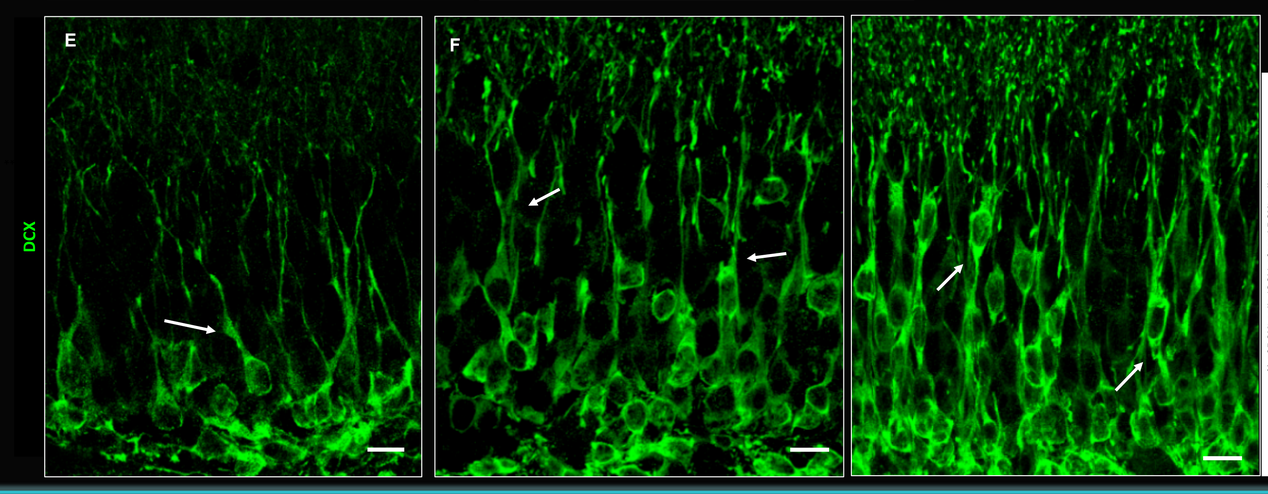


(A) Representative confocal-microscopy images of of 25-day old WT (up) and DS (down) mice after immunostaining for GFAP (green), Ki67 (red) and S100β (blue). NSCs, identified as GFAP+/S100β- cells with radial morphology and soma located in the SGZ, divided with higher frecuency in DS mice. The absolute number **(C)** and percentage **(D)** of dividing (Ki67+) NSCs was significantly increased in DS mice. In addition, in DS mice NSCs showed a reactive-like phenotype **(B)**. The thickness of the primary apical processes **(E)**; as well as the number of primary processes (those emerging directly from the soma) **(E)** and secondary processes (those emerging from the primary processes) were significantly increased in NSCs in DS mice. n = 8 for WT and n = 5 for DS. Data were analysed by Student's t-test, data were subjected to log10-simple transformation when homocedasticity test was not passed. *p= *p < 0.05, **p < 0.01, ***p < 0.001. Bars show mean ± SEM. Dots show individual data. ML, molecular layer. GCL, granule cell layer. SGZ, subgranular zone. Scale bar is 20 µm in A and 10µm in B.

3. ABERRANT NEUROGENESIS IS INDUCED in the DG of DS MICE

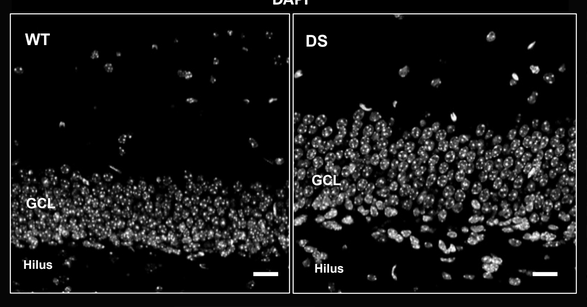


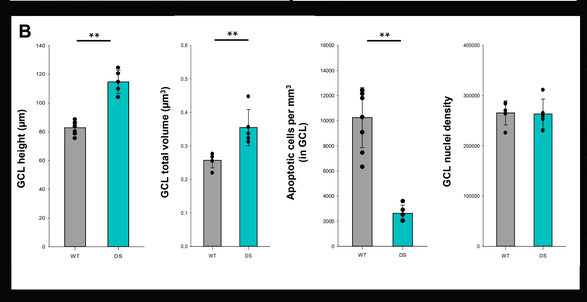




(A) Representative confocal-microscopy images of 25-day old WT (up) and DS (down) mice after immunostaining for doublecortin (DCX), a specific marker for neuroblast (immature neurons) (green) and also stained for DAPI (blue). Neurogenesis was abundant in the DG of WT mice but is further increased in DS mice. The density of DCX+ cells in the GCL, hilus and GCL+hilus is significantly higher in DS mice **(B)**. Further, neuroblasts were distributed differently in the DS mice (right) versus the WT ones (left) **(C)**; In DS mice DCX+ migrated to upper layers closer to the molecular layer, whereas in the WT mice they were localized mainly in the SGZ and lower layers (closer to the hilus) **(D)**. In addition, DS mice presented features of aberrant neurogenesis: in contrast to WT mice **(E)**, neuroblasts in the DS mice presented V-shaped dendrites (arrows, two branches emerging directly from the soma or from the main one bifurcating within the first 10 µm) and basal dendrites **(F)**. The proportion of DCX+ cells with V-shaped dendrites and with basal dendrites was significantly higher in DS mice **(G)**. All these results suggest aberrant neurogenesis is induced in the DG of the DS mice. n = 8 for WT and n = 5 for DS. Data were analysed by Student's t-test, data were subjected to log10-simple transformation when homocedasticity test was not passed. *p= *p < 0.05, **p < 0.01, ***p < 0.001. Bars show mean ± SEM.. Dots show individual data. ML, molecular layer. GCL, granule cell layer. SGZ, subgranular zone. Scale bar 20 µm in A, C and 10 µm in E and F.

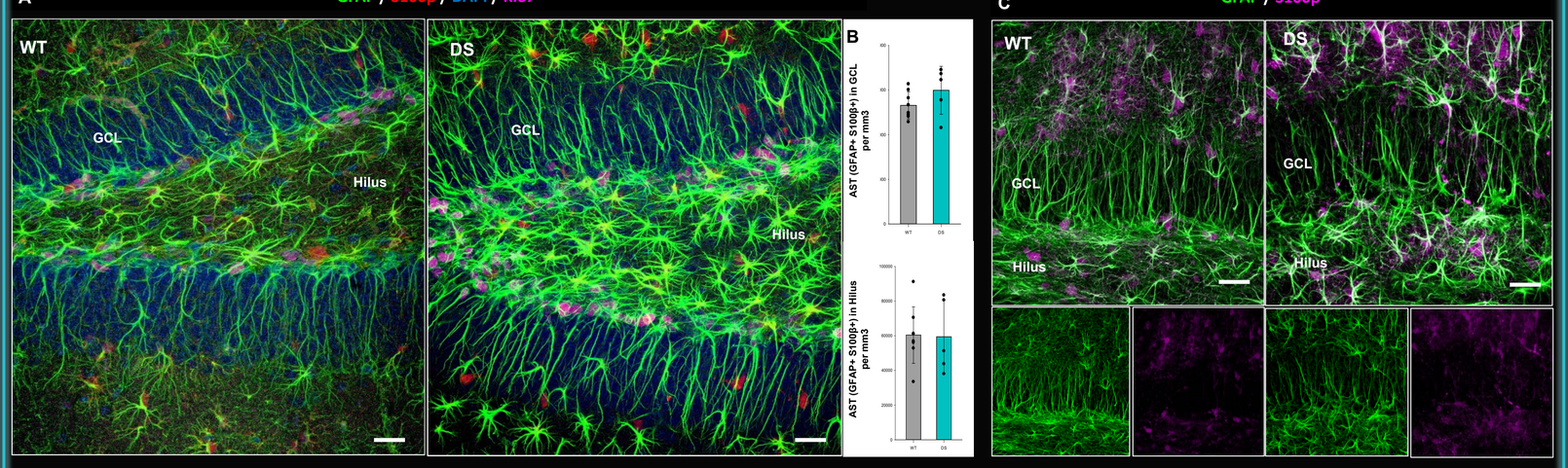
4. CELL DEATH IS DECREASED in the DG of DS MICE





(A) Representative confocal-microscopy images of DAPI staining (white) in 25-day old WT (left) and DS (right) mice. GCL thickness is markedly increased in DS mice **(B).** Quantification of the GCL height and volume showing a statistically significant increase in both cases. Cell density, however, was not changed. Furthermore, we measure cell death by counting apoptotic cells (pinocytic nuclei) in GC and found significantly fewer cells in the DS mice. Data were analysed by Student's t-test, data were subjected to log10-simple transformation when homocedasticity test was not passed. *p= *p < 0.05, **p < 0.01, ***p < 0.001. Bars show mean ± SEM. Dots show individual data. ML, molecular layer. GCL, granule cell layer. SGZ, subgranular zone. Scale bar 20 µm.

5. ASTROGLIOSIS IS PRESENT in the DG of DS MICE



(A) Representative confocal-microscopy images of the DG of 25-day old WT (left) and DS (right) mice after immunostaining for GFAP (green), Ki67 (magenta), S100β (red) and DAPI (blue). Astrocytes were identified as GFAP+/S100β+ cells. We observed that no significant differences in total numbers of astrocytes either in the GCL or the hilus **(B)**. However, GFAP immunostaining flurescence intensity wss markedly higher in DS compared to WT **(C)**. n = 8 for WT and n = 5 for DS. Data were analysed by Student's t-test, data were subjected to log10-simple transformation when homocedasticity test was not passed. *p= *p < 0.05, **p < 0.01, ***p < 0.001. Bars show mean ± SEM. Dots show individual data. Scale bar 20 µm in A and C.

CONCLUSIONS (at P25)

1. Proliferation is increased in DS mice. Also NSCs divide more and show a reactive phenotype in the DG of DS.
2. Generation of new neurons is increased in the DG of DS mice, however neurogenesis is aberrant: new neurons migrate abnormaly and their morphology is altered.
3. The volume of the GCL is larger, with maintained cell density, in DS mice .
4. There is less apparent cell dead in the DG of DS mice.
5. Astrogliosis is also present in DS mice.

Founding

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