Loss of post-natal neurogenesis produces a specific impairment of incidental contextual learning

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Introduction

Post-natally generated granule cells play an important role in the development and function of the dentate gyrus. In adulthood, the continual generation of new cells provides a pool of immature neurons that show enhanced synaptic plasticity and preferential integration into memory circuits. Their exact role in learning and memory processes is still unclear, however, due to large discrepancies in the behavioral literature, particularly in studies of contextual fear conditioning.

The present study sought to test the hypothesis that adult generated granule cells, due to their enhanced capacity for synaptic plasticity, may be critically involved in "incidental" contextual learning, where learning about the context occurs in the absence of the shock.

Post-natal neurogenesis was completely abolished via Cre-recombinase conditional deletion of DNA methyltransferase-1 (DNMT1) in glial fibrillary acidic protein (GFAP) positive cells to produce DNMT1 conditional knock-out mice (DNMT1 CKO). These mice have normal embryonic neurogenesis but a complete loss of post-natal neurogenesis.

DNMT1 CKO mice

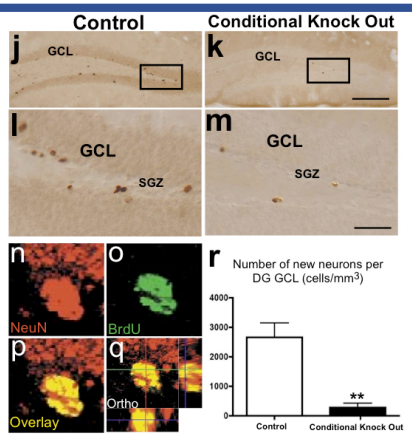

Figure 1: ***Conditional deletion of DNMT1 in GFAP+ cells produces a 90% loss of neurogenesis in the sub-granular zone of the dentate gyrus.*** (j-k) BrdU labelling in control and DNMT1 CKO mice 14 days after 7 twice daily injections of 200 mg/kg BrdU (n-o) Colabelling of BrdU and NeuN labelling (r) Quantification of BrdU labelling in control and DNMT1 mice. n= 6 per group

DNMT1 CKO mice show a specific impairment in incidental contextual fear

Standard vs. Incidental Contextual Fear

Standard Context Test

Animals were placed in the conditioning chamber and presented with a .75 mA foot-shock 5 minutes later. They were returned to the conditioning chamber 24 hours later for an 8 minute context test.

Incidental Contextual Fear

On Day 1 animals were given a 5 minute pre-exposure or no pre-exposure to the conditioning chamber. On Day 2 animals were placed in the conditioning chamber and given a .75 mA foot-shock 10 seconds later. They were then given an 8 minute context test on Day 3. In this procedure non-pre-exposed animals exhibit the immediate shock deficit: e.g. they acquire very little context fear. Pre-exposure to the conditioning chamber, prior to the immediate shock, however, rescues the immediate shock deficit because it allows the animal time to explore and form a contextual representation that can then be retrieved and associated with the immediate shock. The form of learning that occurs during the pre-exposure is referred to as incidental contextual learning as it occurs in the absence of specific motivating stimuli such as the foot-shock.

Standard Context Test

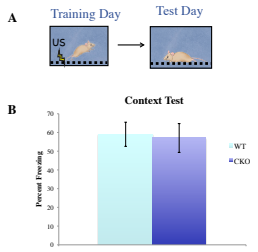

Figure 3: ***DNMT1 CKO mice show normal contextual fear in a standard task.*** (a) Experimental Design (b) Percent freezing in the 8 minute context following standard context training with a 5 minute placement to shock interval. No significant differences or sex interactions were observed. WT, n = 16, CKO, n = 14

Incidental Contextual Learning

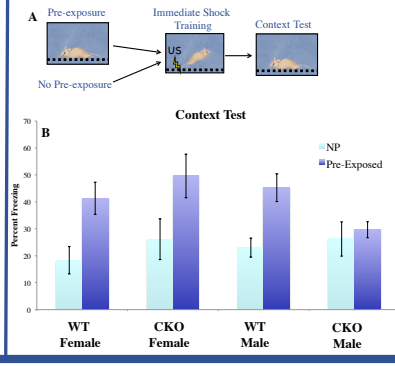

Figure 3: ***DNMT1 CKO mice show a sex-dependent impairment in incidental contextual learning.*** (a) Experimental Design (b) Percent freezing in the 8 minute context test following 5 minutes of context pre-exposure or no pre-exposure (NP) and immediate shock training. A significant sex interaction was observed in this study, therefore the results are broken down by sex.

NP animals show the immediate shock deficit. Pre-exposure rescued this deficit in all groups except male CKO's.

Female WT NP, n = 12; Female WT Pre, n = 13; Female CKO NP, n = 9; Female CKO, n = 9; Male WT NP, n = 15; Male WT Pre, n = 13;

DNMT1 cKO mice show normal acquisition and generalization of contextual fear

Contextual fear acquisition and generalization:

Animals were placed in the conditioning chamber and presented with a .65 mA foot-shock 3 minutes later. This was repeated for 5 days with freezing being measured in the 3 minutes preceding the shock on each day. Generalization of fear to a novel context was tested over two days. The training context consisted of: alternating size grid floor, Windex scent, no background fan, curved back, cleaned with 70% ethanol. The novel context consisted of: uniform grid floor, Simple Green scent, background fan, flat back, cleaned with 70% isopropyl alcohol. On Day 8 the odors used to distinguish the training from the novel context were reversed.

Contextual Fear Acquisition

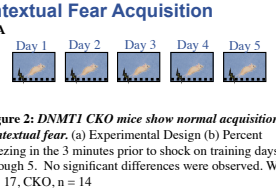

Figure 2: ***DNMT1 CKO mice show normal acquisition of contextual fear.*** (a) Experimental Design (b) Percent freezing in the 3 minutes prior to shock on training days 2 through 5. No significant differences were observed. WT, n = 17, CKO, n = 14

Generalization

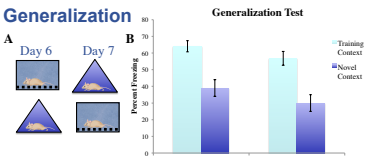

Figure 3: ***DNMT1 CKO mice show normal generalization of contextual fear.*** (a) Experimental Design (b) Percent freezing in the 3 minute context tests in the training and novel contexts. No significant differences were observed. WT, n = 17, CKO, n = 14

Sensitivity to change in odor

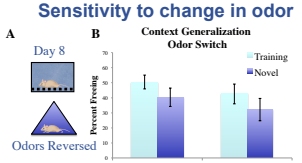

Figure 4: ***DNMT1 CKO mice show use of odor cues in discriminating contexts.*** (a) Experimental Design (b) Percent freezing in the 3 minute context tests in the training and novel contexts with odors reversed. No significant differences were observed. WT, n = 17, CKO, n = 14

Exploratory Activity

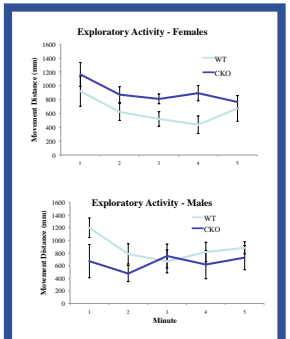

Figure 3: ***DNMT1 CKO mice show sex dependent changes in exploratory activity.*** (a) Female CKO's show increased exploration WT, n = 5; CKO, n= 12; (b) Male CKO's show decreased exploration, WT, n = 5; CKO, n = 4

Conclusions

- Loss of post-natal neurogenesis in DNMT1 CKO mice results in:
  - Normal acquisition of contextual fear in standard tasks
  - Normal discrimination between two distinct contexts and normal use of odor cues in discrimination between contexts
  - A sex-dependent impairment in incidental contextual learning, where learning about the context occurs in the absence of shock
- Taken together, these results suggest that loss of post-natal neurogenesis produces a specific impairment of incidental contextual learning. The enhanced plasticity of immature adult generated granule cells may therefore be necessary for contextual learning under conditions of low stress or motivation but the less plastic mature population may be sufficient under conditions of higher motivation.

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