Examining the role of H3K4 methylation in the hippocampal memory formation and in the pathogenesis of Alzheimer's disease.

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Introduction

Histone H3 lysine 4 methylation (**H3K4me**) is one of the histone modification mechanisms regulating gene transcription. (Particularly tri-methylation of H3K4 (H3K4me3) it is highly enriched at transcription start sites and greatly associated with active transcription[1].

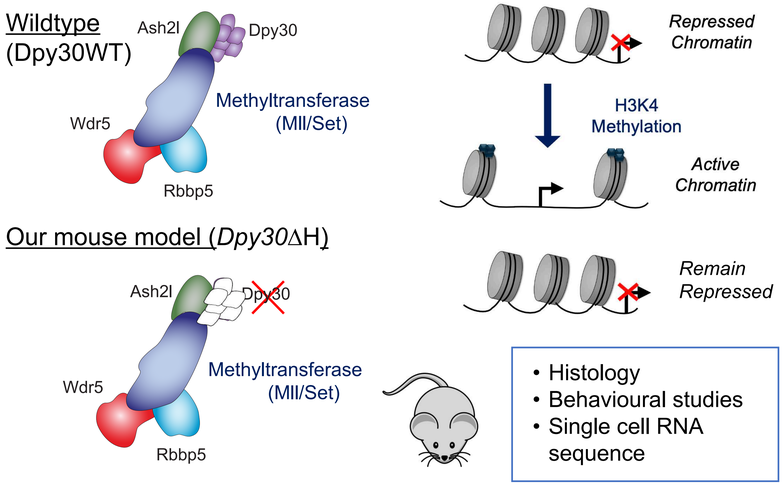
Recently, **H3K4me** has emerged as a key epigenetic marker involved in a process of hippocampal memory formation.

- **H3K4me** was significantly up-regulated in the CA1 region of hippocampus 1 hour after induction of contextual fear conditioning in rats[2].
- A decrease in nuclear **H3K4me** concomitant with increased abnormal cytoplasmic **H3K4me** was observed in the hippocampal neurons of Alzheimer's disease donors[3].

We hypothesize that H3K4 methylation is required for proper formation of hippocampus-dependent memory and its de-regulation is involved in the pathogenesis of Alzheimer's disease.

Objective and Method

To determine if **H3K4 methylation** is required for transcriptional activation of memory genes in the hippocampus, we disrupted catalytic activity of **methyltransferase** in the CA1 pyramidal neurons of adult mice.



- Histology
- Behavioural studies
- Single cell RNA sequence

Is **H3K4 methylation** altered in the hippocampal neurons in Alzheimer's disease?

Post-mortem hippocampal samples of AD vs healthy donors
- Histology and Western Blot

Loss of H3K4me3 appears by 3 months of age

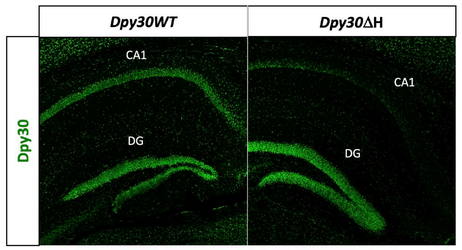


Fig 1: Immunofluorescent (IF) staining of DPY30 indicates **CA1 specific deletion of Dpy30 in the hippocampus of 2 months-old mice.**

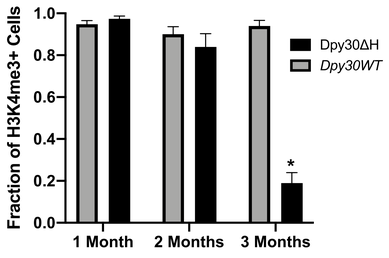
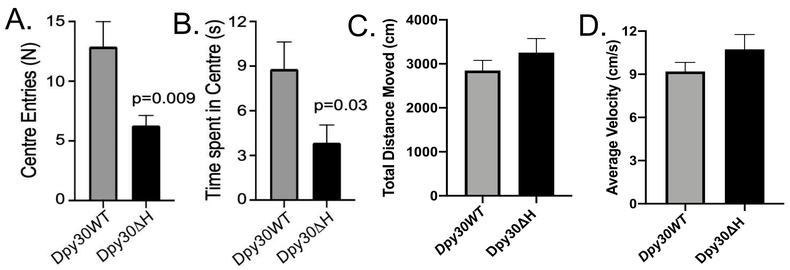


Fig 2: Quantification of H3K4me3 positive cells in the CA1 hippocampal regions of 1-3 months old Dpy30WT and Dpy30ΔH mice. **Significant reduction of H3K4me3 is delayed to 3 months of age.** (↓ Dpy30: by 2 months of age)

Anxiety-like behaviour in *Dpy30*ΔH mice



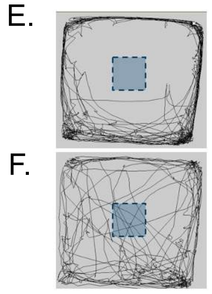


Fig 3: ***Dpy30*ΔH mice exhibit anxiety-like behaviour but have normal locomotory coordination** in open field test. (A) Number of entries to the centre arena. (B) Duration of time in the centre arena. (C) Total distance travelled in the entire arena during 5 minutes of measurement. (D) Average velocity travelled. (E-F) The trajectory plot of (E) *Dpy30*ΔH and (F) Dpy30WT [3-6 months old, n=10/group].

Behavioural inflexibility in *Dpy30*ΔH mice

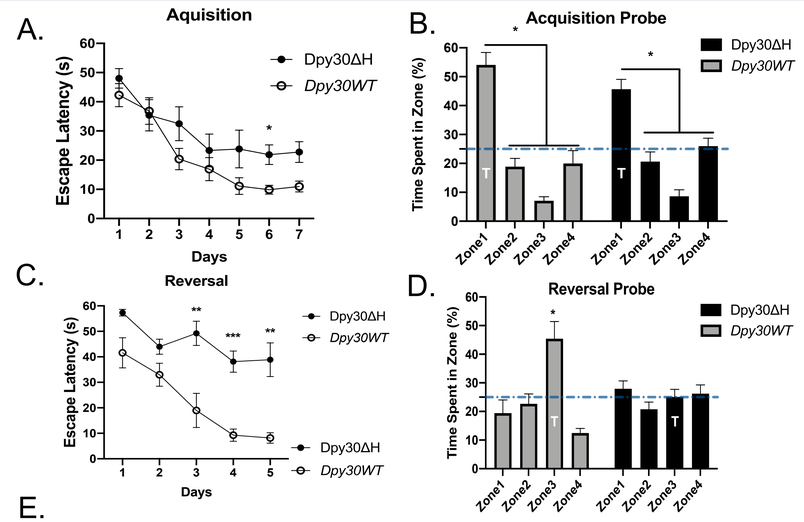


Fig 4: Morris water maze (MWM) test results indicate that ***Dpy30*ΔH mice require more time finding hidden platform** and **have significantly impaired reversal memory.**

(A) Escape latency during acquisition training. (B) Zone of preference after 7 days of acquisition training. (C) Escape latency during reversal training. (D) Zone of preference after 5 days of reversal training. (E) Mean velocity of swimming throughout MWM study.

(Preliminary) Reduced level of H3K4me3 in AD

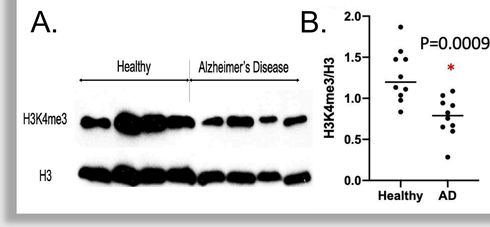


Fig 5: **Reduced level of H3K4me3 observed in hippocampal homogenate of AD sample.** (A) Immunoblotting result, (B) Quantification [n=10/group]

Conclusion and Future Plan

- There is a one-month delay between significant loss of *Dpy30* and H3K4me3 in the hippocampus of *Dpy30*ΔH mice.
- *Dpy30*ΔH mice exhibit anxiety-like behaviour and have significant deficits in behavioural flexibility with normal locomotor activity.
- Significantly lower level of H3K4me3 was observed from the hippocampal tissue homogenate of Alzheimer's disease human samples compared to age- and sex-matched healthy control.

▶ Perform Sc-RNA seq to examine if loss of H3K4me affects memory-associated gene expression and perform IF on clinical sample

1) Takahashi, Y.H *et al.* 2011. PNAS **108**:5, 2) Gupta, S. *et al.* 2010. J Neurosci **30**, 3) Mastroeni, D *et al.* 2016. Neurobiol Aging **36**:12