Neuronal Primary Cilia Regulate Hippocampal Neuronal Priming and Trace Memory Formation

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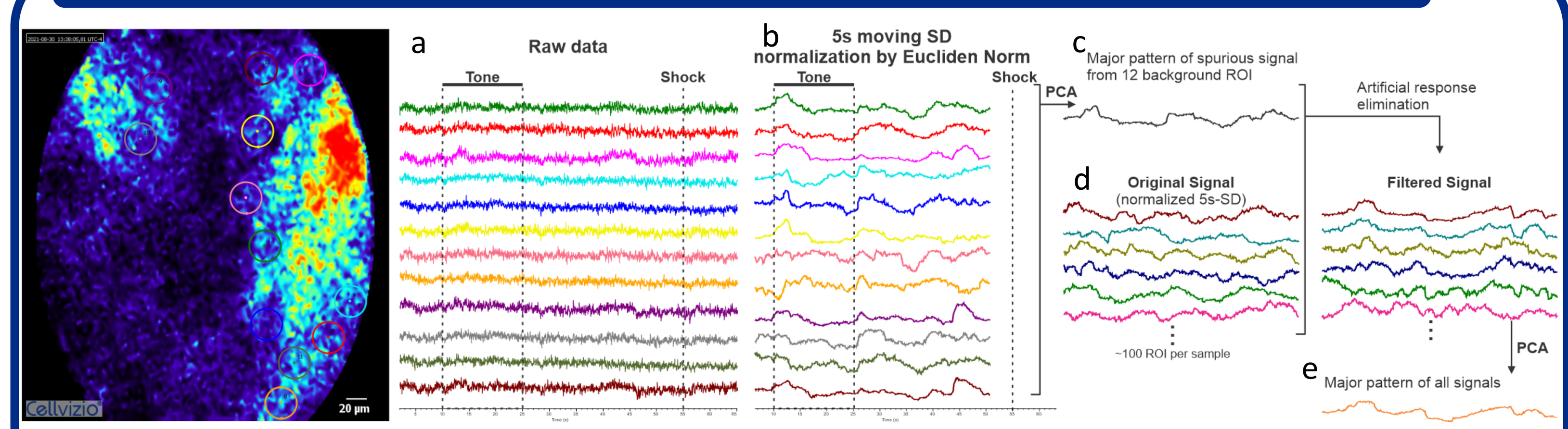
Abstract

Memory is thought to be encoded by sparsely distributed neuronal ensembles in memory-related regions. Using *in vivo* calcium imaging, we have previously discovered that a small fraction of "primed neurons" in the hippocampus actively engage in trace memory information by forming burst synchronization. Our imaging system allows for direct visualization of neuronal activity in freely behaving animals. However, its application is limited by some motion artefacts and low signal-to-noise ratio (SNR). We have developed a robust computational algorithms to eliminate artificial burst, numerically define primed neurons, and compute memory-associated neuronal synchronization.

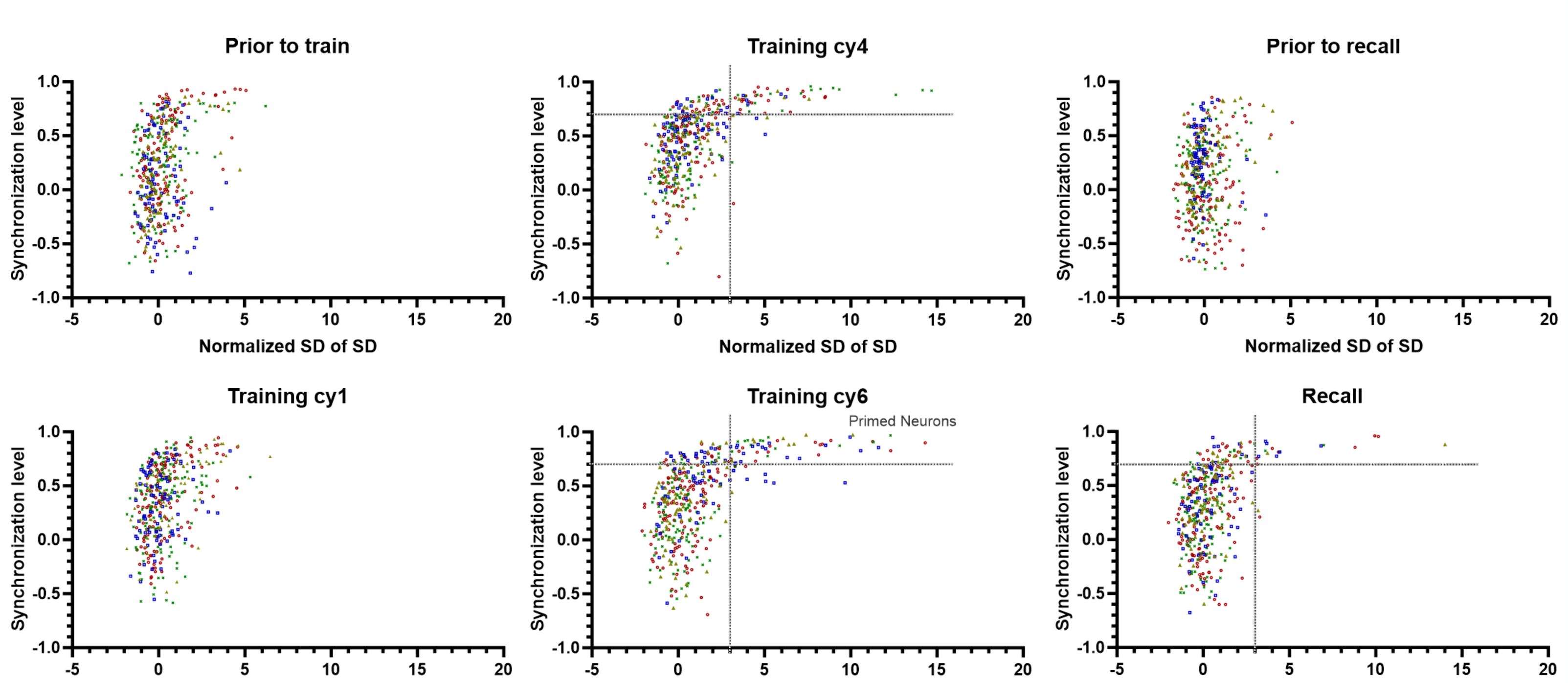
Primary cilia are microtubule-based sensory organelles present in most vertebrate cells including neurons. The deflection of neuronal cilia is associated with numerous neurological disorders like intellectual disability, cognitive impairment, and neurodegeneration. It is unclear that how neuronal primary cilia regulate the activity of hippocampal neurons and affect hippocampus-dependent memory formation. We utilized two loss-of-function mouse models a) globally ablated primary cilia in adult mice (IFT88 global KO) and b) shortened primary cilia in the adult forebrain (Arl13b global KO). We subjected these mice to trace fear conditioning tests in conjunction with *in vivo* calcium imaging study. Here we show that both of KOs display a markedly impaired learning ability than their littermate Controls. The overall activity level of hippocampal neurons of KOs is significantly decreased compared to Controls. Moreover, the burst synchronization following fear conditioning is drastically suppressed. These data suggest that neuronal primary cilia regulate hippocampal neuronal priming.

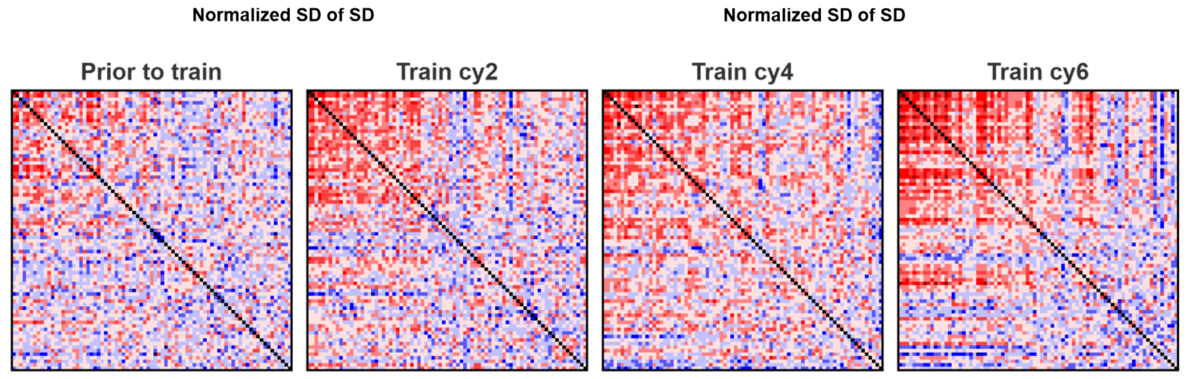
Method: Eliminate moving artefacts and extract neuronal signals



In vivo calcium imaging is a neuroimaging technique that allows for the simultaneous observation of neuronal population activity. We utilize a cutting-edge system which records calcium imaging in free-moving mice. The free behavior allows multiple advanced behavioral tests than head-mounted calcium imaging system. However, it requests a better analysis method to extract signal from noise and artefacts. **Fig. 1. a)** Raw calcium traces from 12 scattered background ROIs. **b)** 5s moving SD trace to reduce random noise, normalization of Euclidean Norm to reduce the effect of intensity. **c)** Principal component analysis (PCA) to extract major background signal pattern (bgs). **d)** compare all calcium traces with bgs trace and eliminate moving artefacts. **e)** The major pattern of all filtered signals will be used for further statistical analysis.

Method: Define primed neurons and compute synchronization





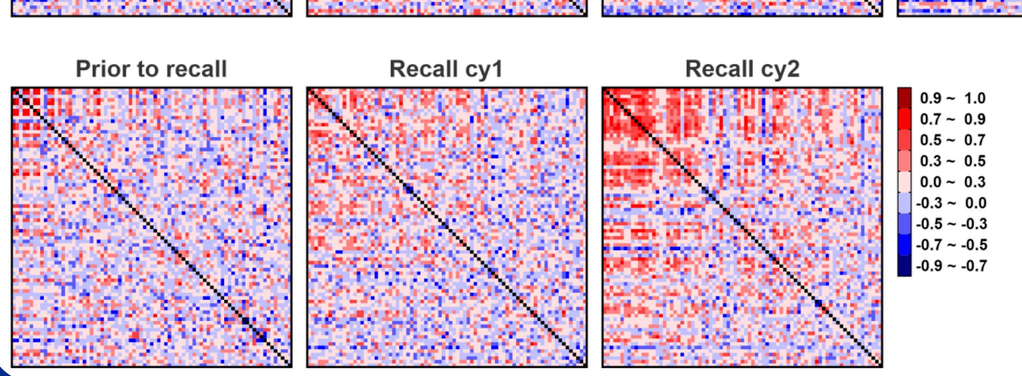
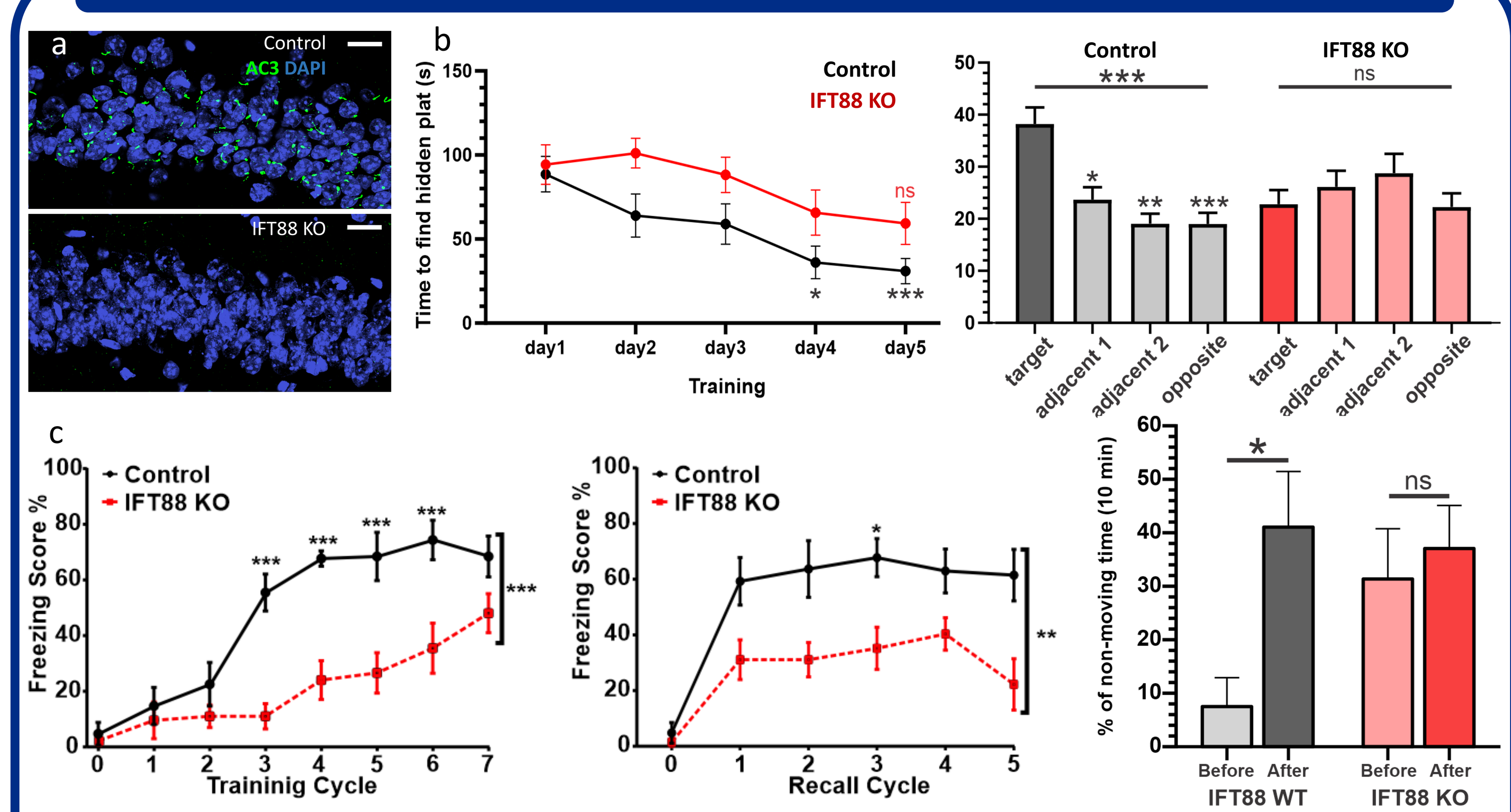
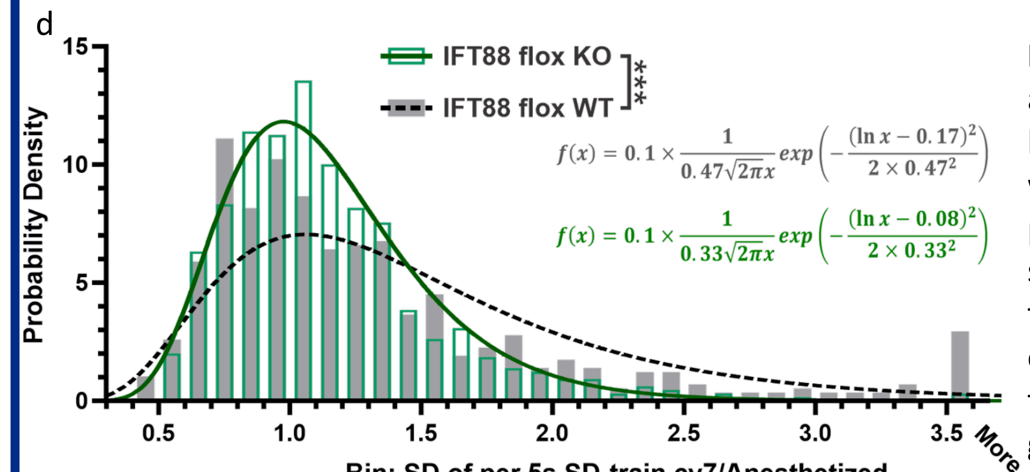


Fig. 2. (Top) Scatter plot of single neuron activity vs synchrony level. X-axis is the SD of time-windowed SD(5s) for every single neurons; Y-axis is synchronization level of same neurons comparing to major activation signal pattern; Different symbols for 4 animals. Most neurons concentered while as a portion of primed neurons start to scale out by training and recall. **Bottom** Heat map of pair-wised correlation during training and recall.

Fig. 3. IFT88 KOs manifest impaired spatial and trace fear learning





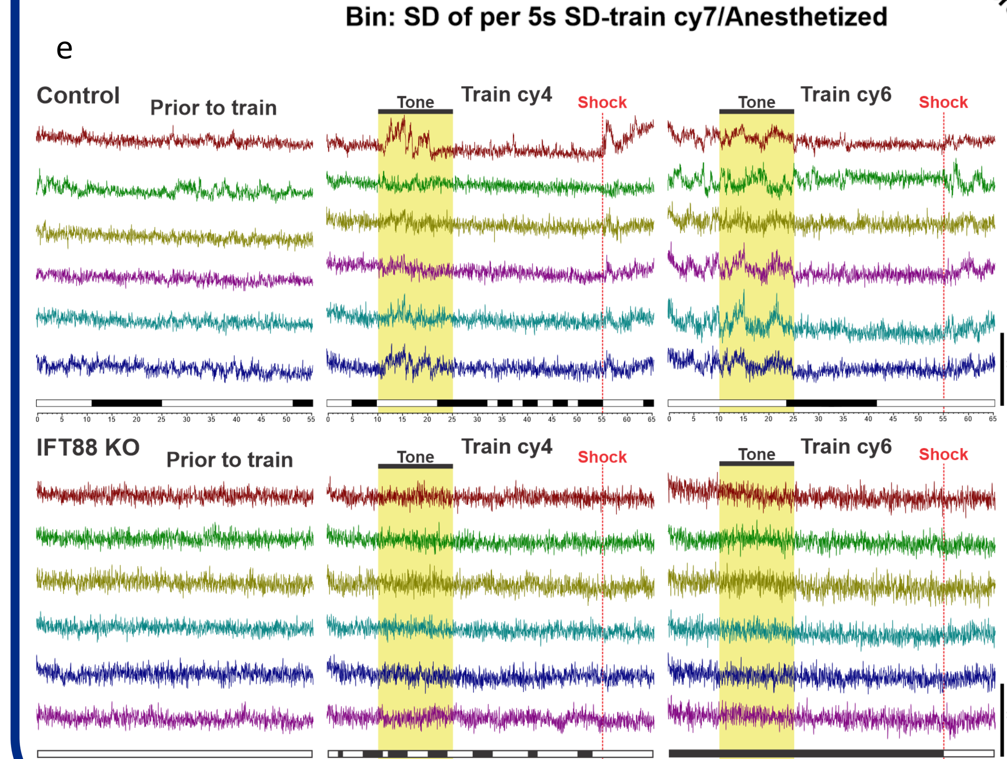
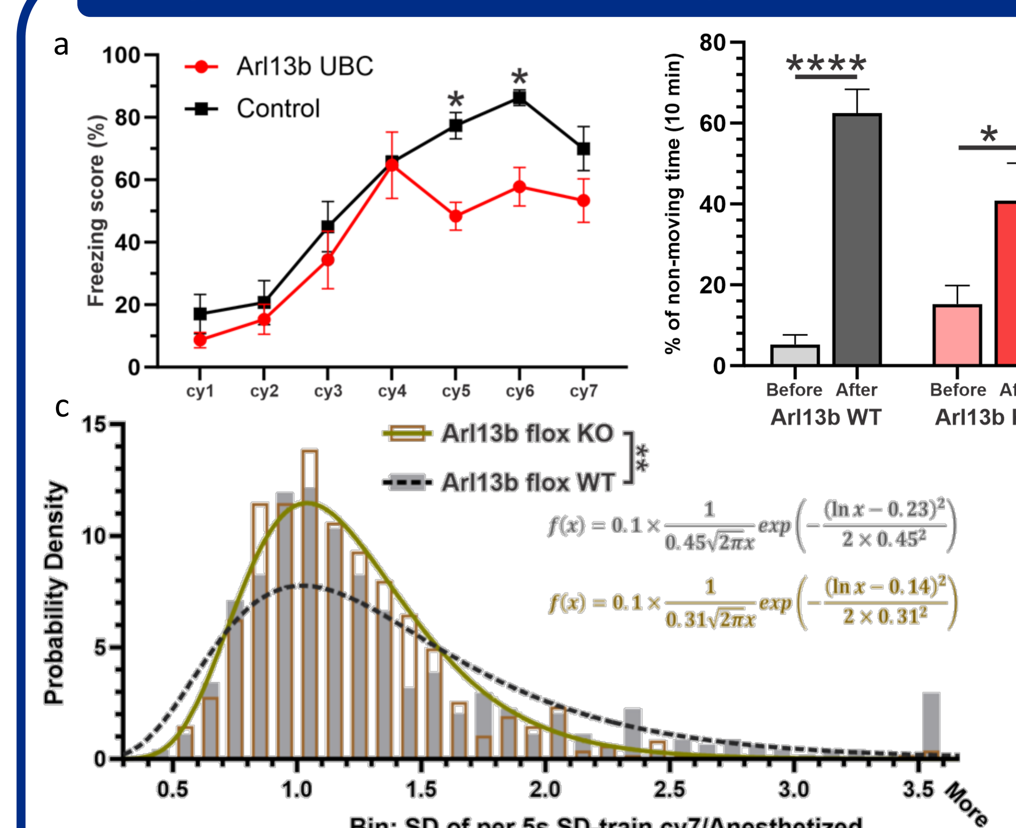
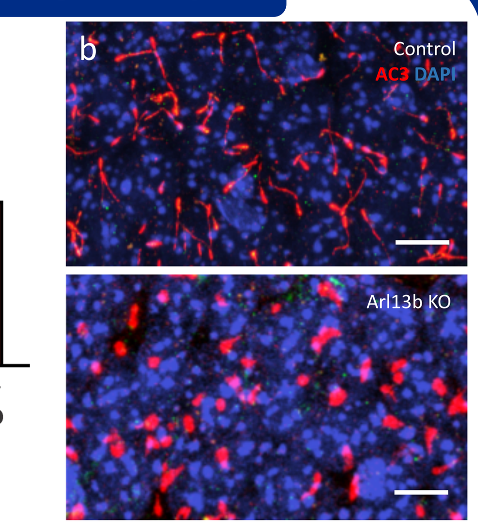
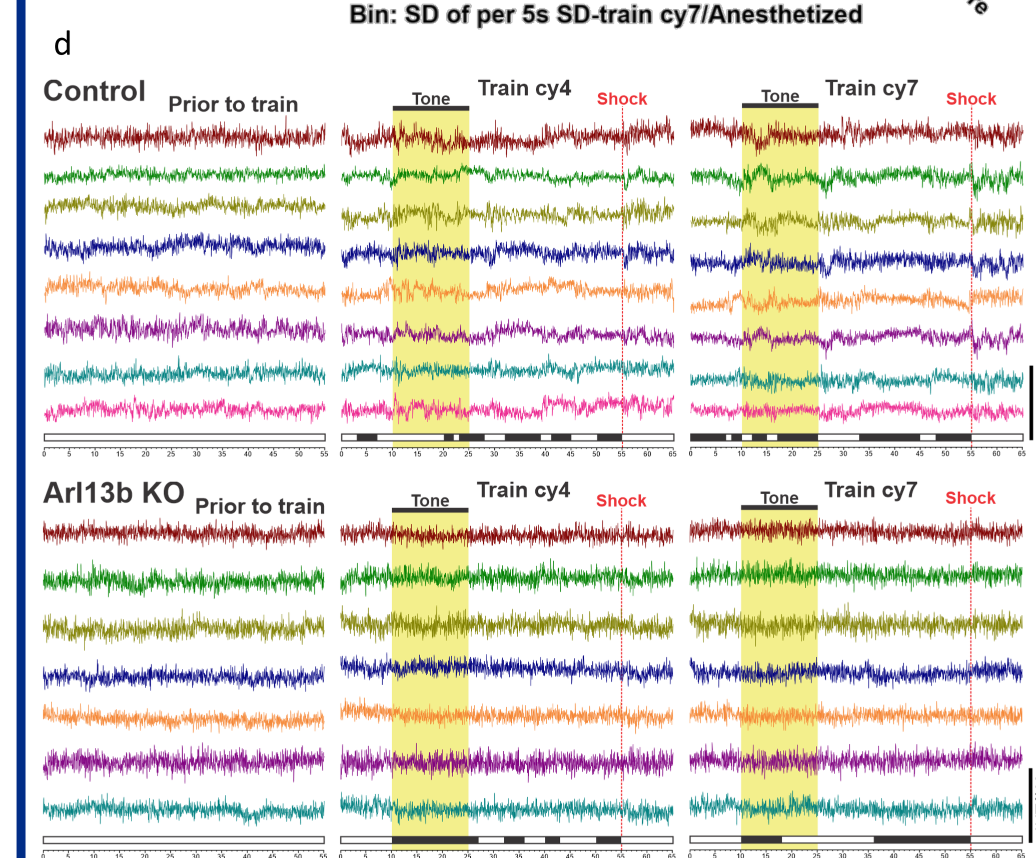


Fig.3. a) IFT88 KO mice have almost no primary cilia in their hippocampus. 20μm. **b)** In Morris water maze test, Control mice performed better in training and spent significant longer time in target zone, whereas IFT88 KOs can't distinguish 4 zones. **c)** In trace fear conditioning test, IFT88 global KOs displayed a markedly reduced freezing than controls during learning and recall. Controls' freezing time increased after training, while as IFT88 KOs show no difference before and after training. **d)** Neuronal activity level distribution. Compared to control, the activity histogram of IFT88 KOs significantly shifted to the left, indicating that the basal activity levels of hippocampal neurons are markedly reduced in IFT88 global KOs. **e)** Representative individual calcium imaging traces of Control and IFT88 global KO. IFT88 KO neurons cannot form synchronized responses.

Fig. 4. Arl13b KOs exhibit impaired trace learning ability







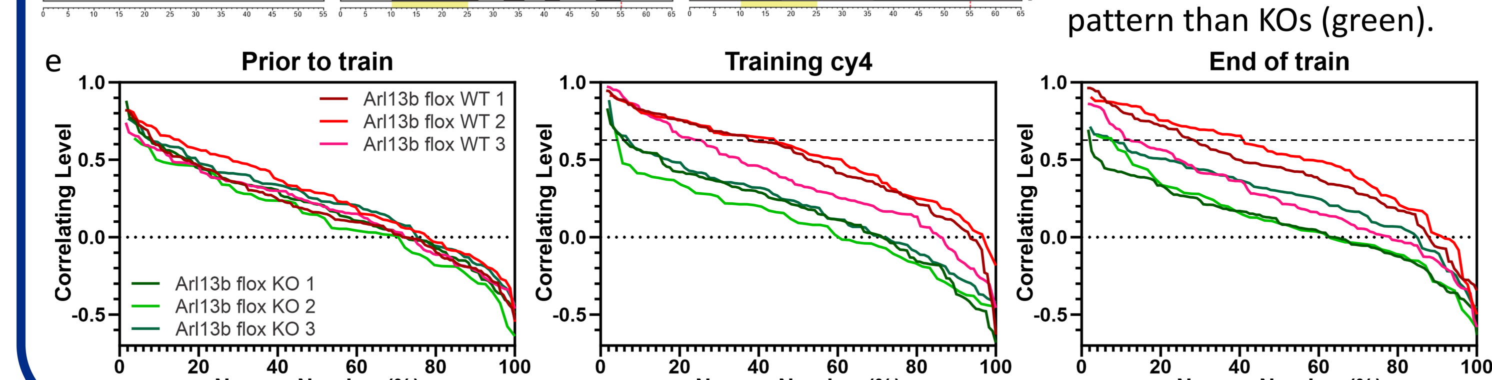


Fig.4. a) In trace fear conditioning, Arl13b global KOs displayed a markedly reduced fear response than controls during learning, and earlier to recover from freezing after training. **b)** Arl13b KO mice have shorter cilia in hippocampus CA1 region. 10μm. **c)** Distribution of hippocampal neuronal activity levels. Compared to controls, the fitting curve of activity histogram of Arl13b KOs shifted to the left. **d)** Representative individual calcium imaging traces of Control and Arl13b global KO. Arl13b KO neurons cannot form a good synchronization correlating with tone and shock. **e)** Correlating level to major activation signal pattern. Controls (red) had more neurons highly correlating (>0.7) with major pattern than KOs (green).

Acknowledgement and Reference

This study was supported by National Institutes of Health Grants K01AG054729, R15MH126317, and R15MH125305 as well as Cole Neuroscience Faculty Research Awards to X.C. *and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the NIH under grant number P20GM113131.*

Zhou Y, Qiu L, Wang H, Chen X (2020). Induction of Activity Synchronization among Primed Hippocampal Neurons out of Random Dynamics is Key for Trace Memory Formation and Retrieval. FASEB J DOI: 10.1096/fj.201902274R