Pcdhβs affects synchronous activity in the hippocampus

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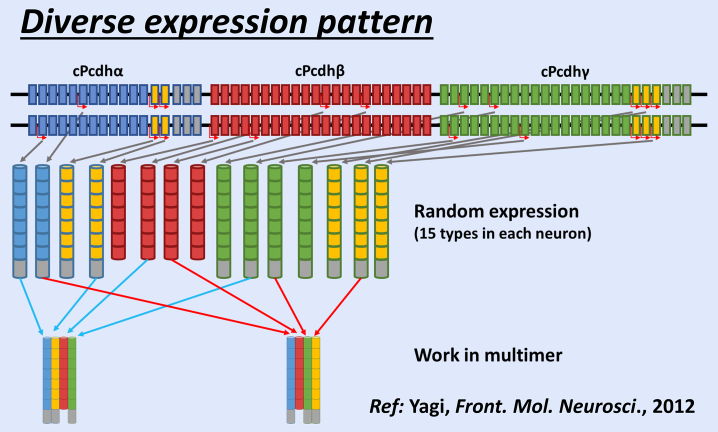
Summary

Background: **Clustered protocadherins (Pcdhs), a large subgroup of adhesion molecules, are important for neural morphology such as axonal projection and dendrite spread. However, little is known how Pcdhs affect neural activity.**
Methods: **Observing neural activity in Pcdhβ-deletion mice with *in vivo* Ca^{2+} imaging, we demonstrate that Pcdhβs affect neural activity in the hippocampus during active state rather than resting state.**
Results: **Pcdhβ-deletion reduced repetitive synchronous activity during novel context exploration and increased large size cell ensembles, which were extracted with non-negative matrix factorization analysis based on synchronous activity. Majority of the large ensembles was rarely activated. Furthermore, larger portion of cells in Pcdhβ-deletion mice were shared between ensembles than wild type mice, which may impair the discrimination of context in contextual fear conditioning task. These phenotypes may result from the involvement of Pcdhβs in adjustment of neural activity through ensemble formation in the hippocampus without affecting cellular activity.**
Conclusion: **Together, Pcdhβs modulate neural ensemble activity in the hippocampus.**

Introduction

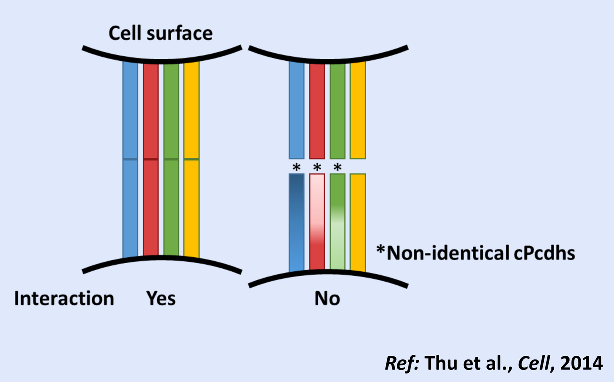
Pcdhs are thought to be important for forming diverse neural networks

Diverse expression pattern



Ref: Yagi, Front. Mol. Neurosci., 2012

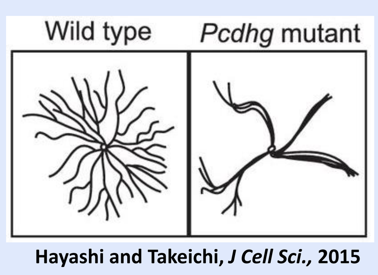
Homophilic interaction



Ref: Thu et al., Cell, 2014

Pcdhs affect neuronal morphology

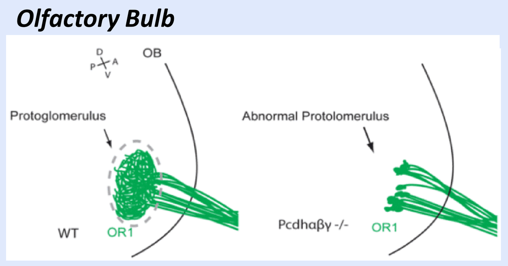
Dendritic spread



Hayashi and Takeichi, J Cell Sci., 2015

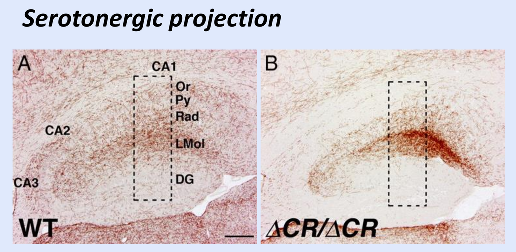
Projection

Olfactory Bulb



Mountoufaris et al., Science, 2017

Serotonergic projection

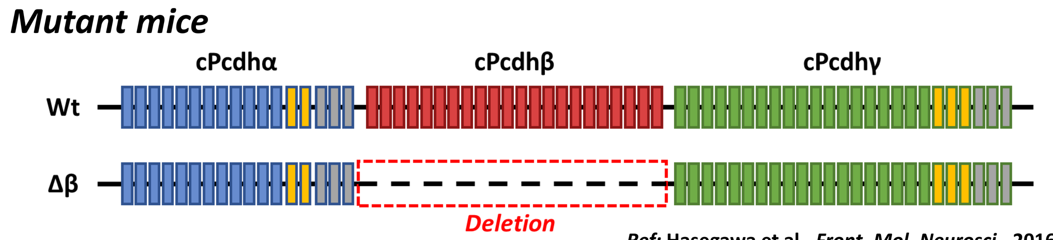


Katori et al., J Neuro Sci., 2009

Question
How do Pcdhs affect neural activity in the hippocampus ?

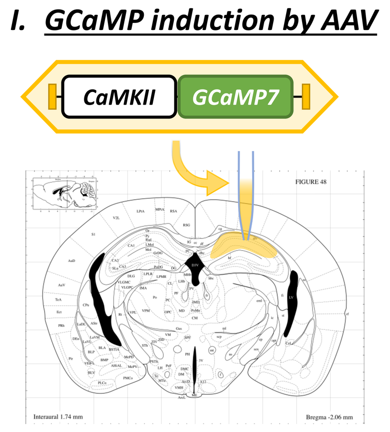
Experiments

Mutant mice

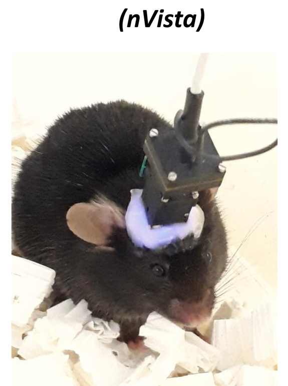


Ref: Hasegawa et al., Front. Mol. Neurosci., 2016

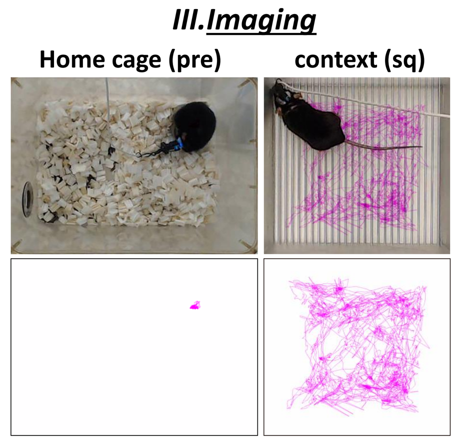
I. GCaMP induction by AAV



II. Setting microscopy (nVista)

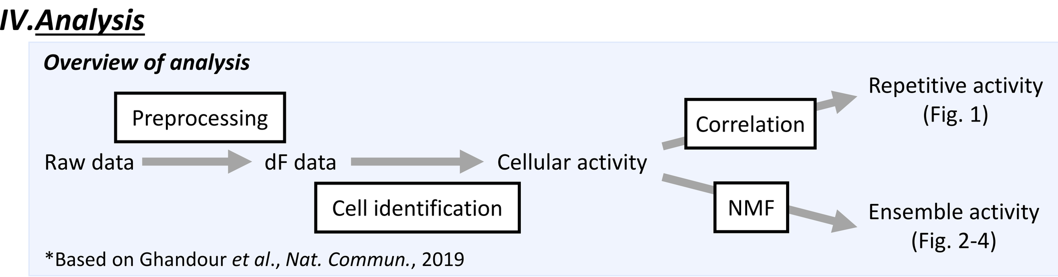


III. Imaging



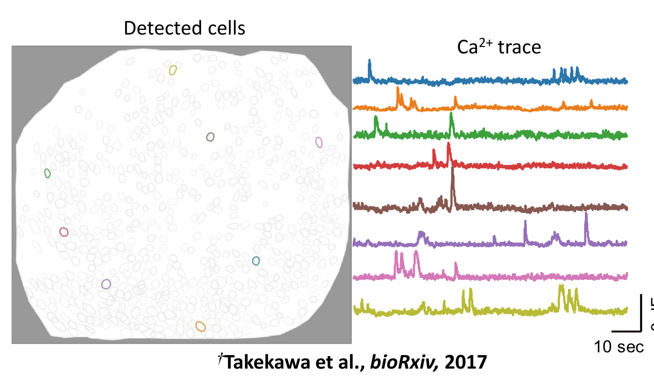
IV. Analysis

Overview of analysis



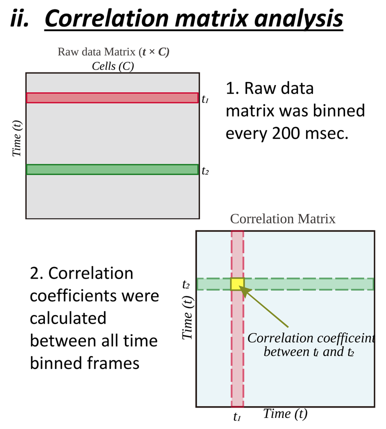
*Based on Ghandour et al., Nat. Commun., 2019

i. Cell identification (HOTARU algorithm[†])

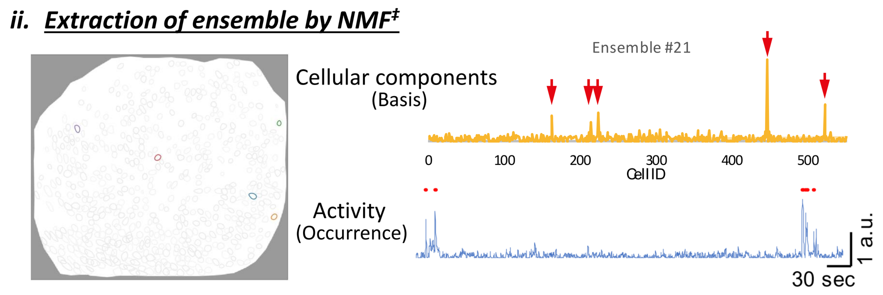


[†]Takekawa et al., bioRxiv, 2017

ii. Correlation matrix analysis



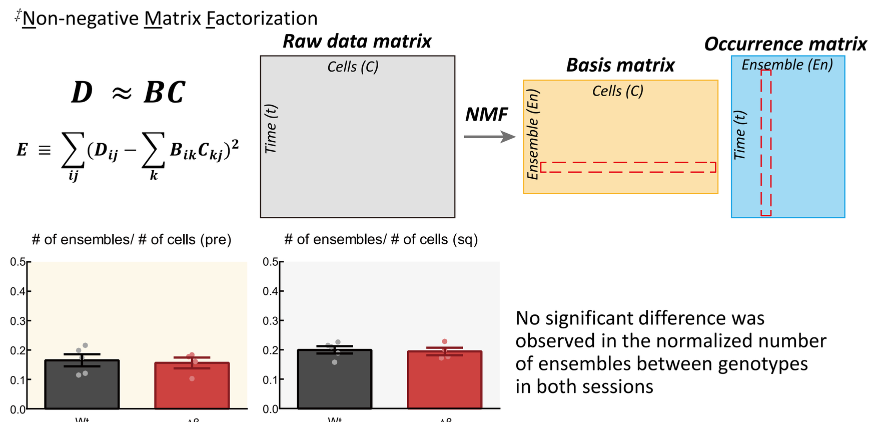
ii. Extraction of ensemble by NMF[‡]



[‡]Non-negative Matrix Factorization

$$D \approx BC$$

$$E = \sum_{ij}\left(D_{ij} - \sum_k B_{ik}C_{kj}\right)^2$$



No significant difference was observed in the normalized number of ensembles between genotypes in both sessions

Pcdhb-deletion decreased repetitive activity during context exploration

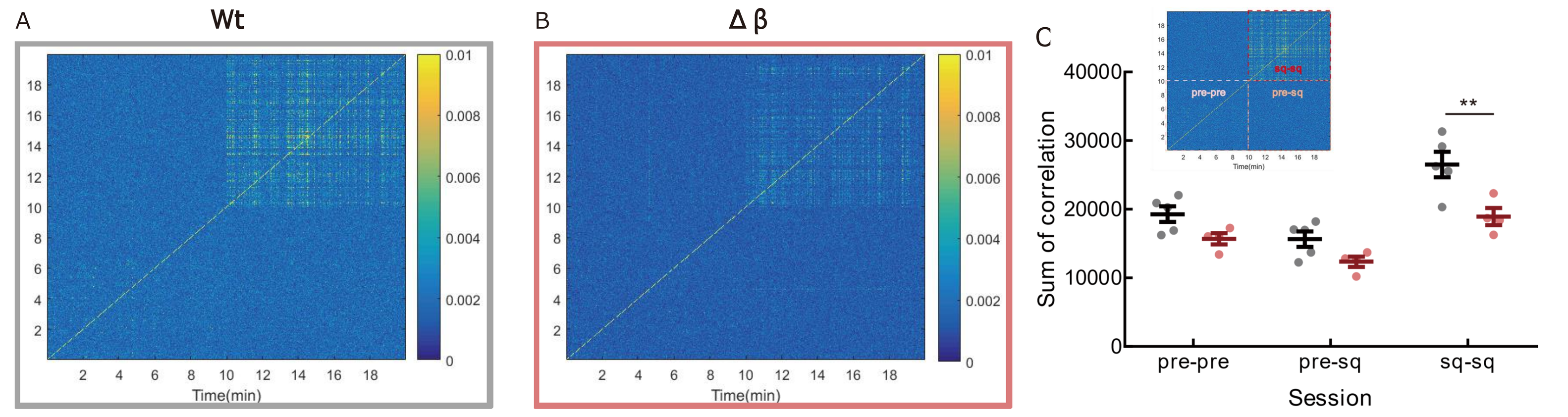


Figure 1. Deletion of cPcdhbs reduced repetitive activity. (A, B) Representative image of correlation matrix (A: Wt, B: Δβ). (C) Summation of correlation coefficient in session by session. n = 5 mice for Wt, n = 4 mice for Δβ. Mean ± S.E.M. $^{**}p < 0.01$, (Adjusted *p*-value of Bonferroni's multiple comparison test).

Pcdhb-deletion increased large ensembles and cells shared between ensembles

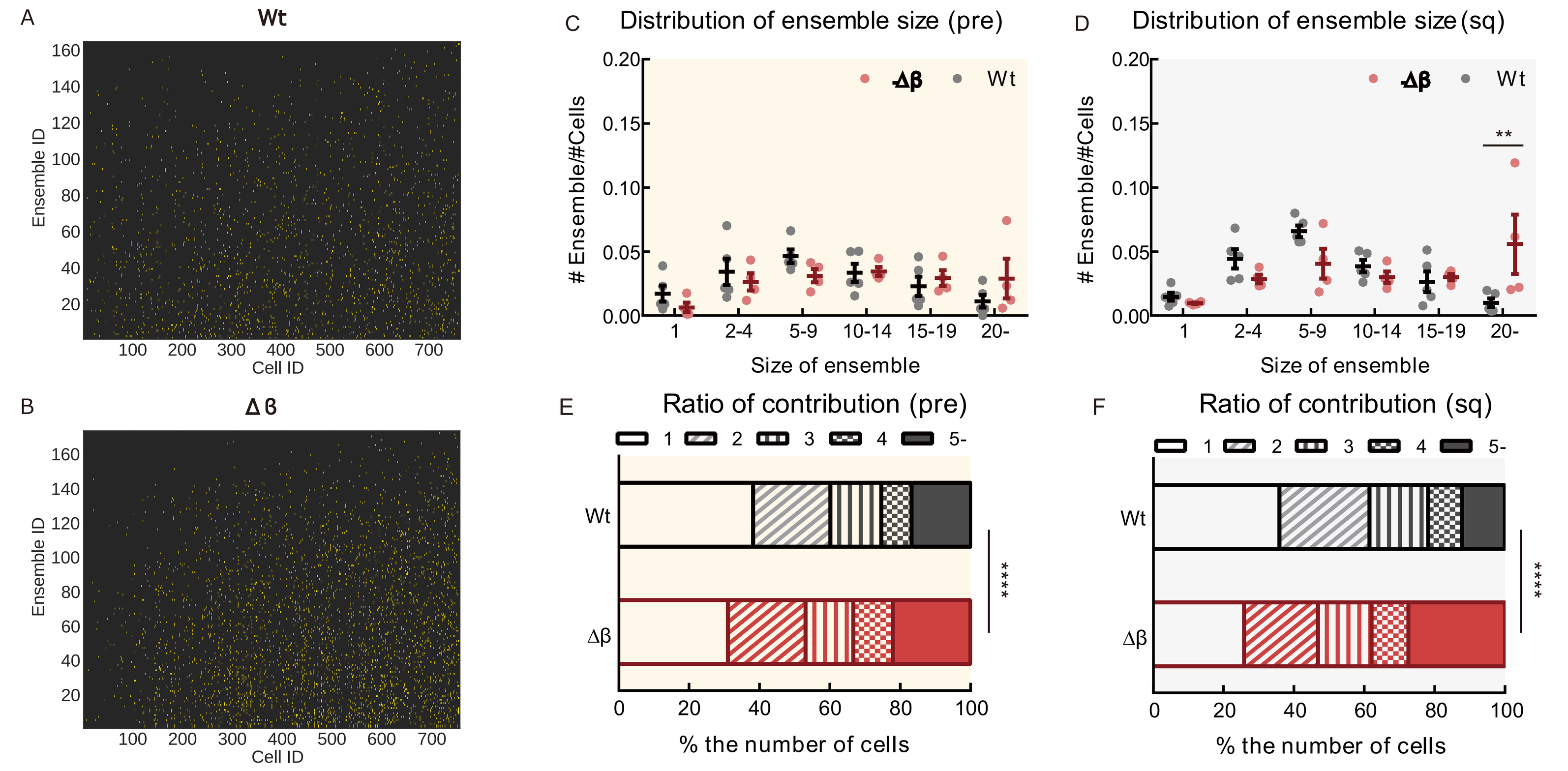


Figure 2. Deletion of cPcdhbs increased large ensembles. (A, B) Representative image of binarized basis matrix. (C, D) Normalized number of ensembles in each ensemble size. n = 5 mice for Wt, n = 4 mice for Δβ, Mean ± S.E.M. $^{**}p < 0.01$ (Adjusted *p*-value of Bonferroni's multiple comparison test). (E, F) Ratio of cells contributing singular (open bar) and plural (pattern or solid bar) ensembles. $^{****}p < 0.0001$ (Chi-square test)

Large ensembles in Pcdhb-deletion mice showed rare activity

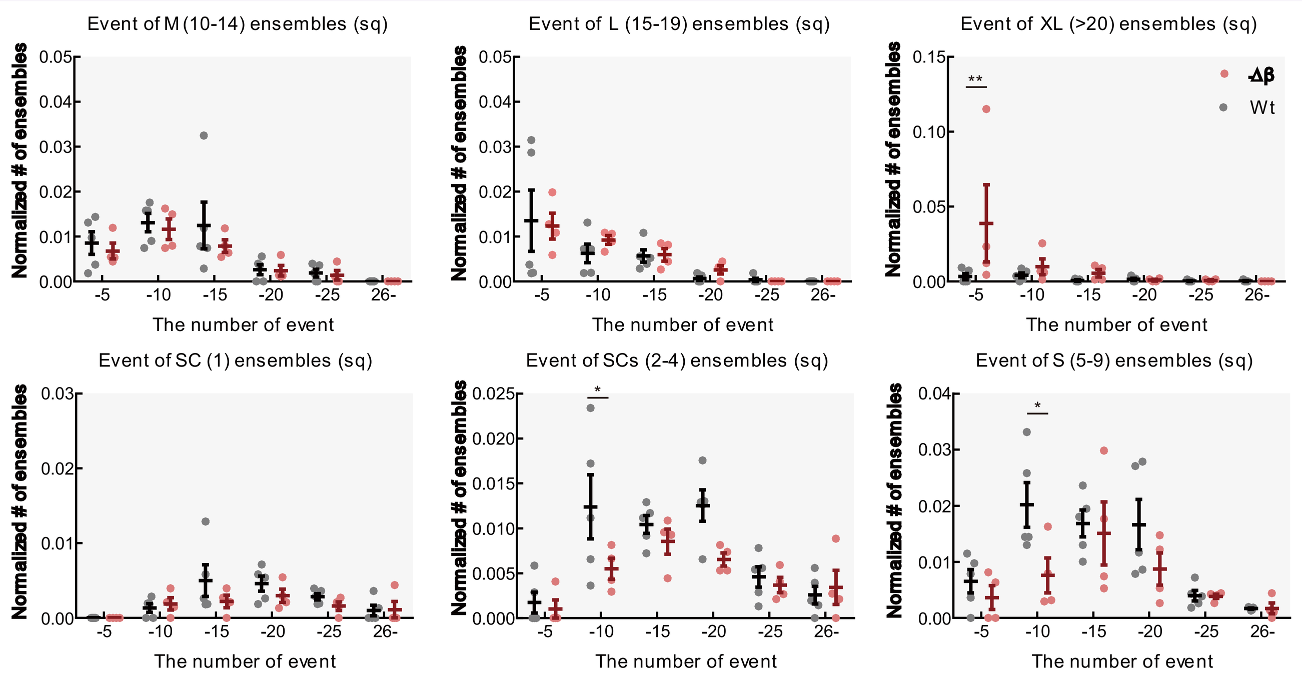
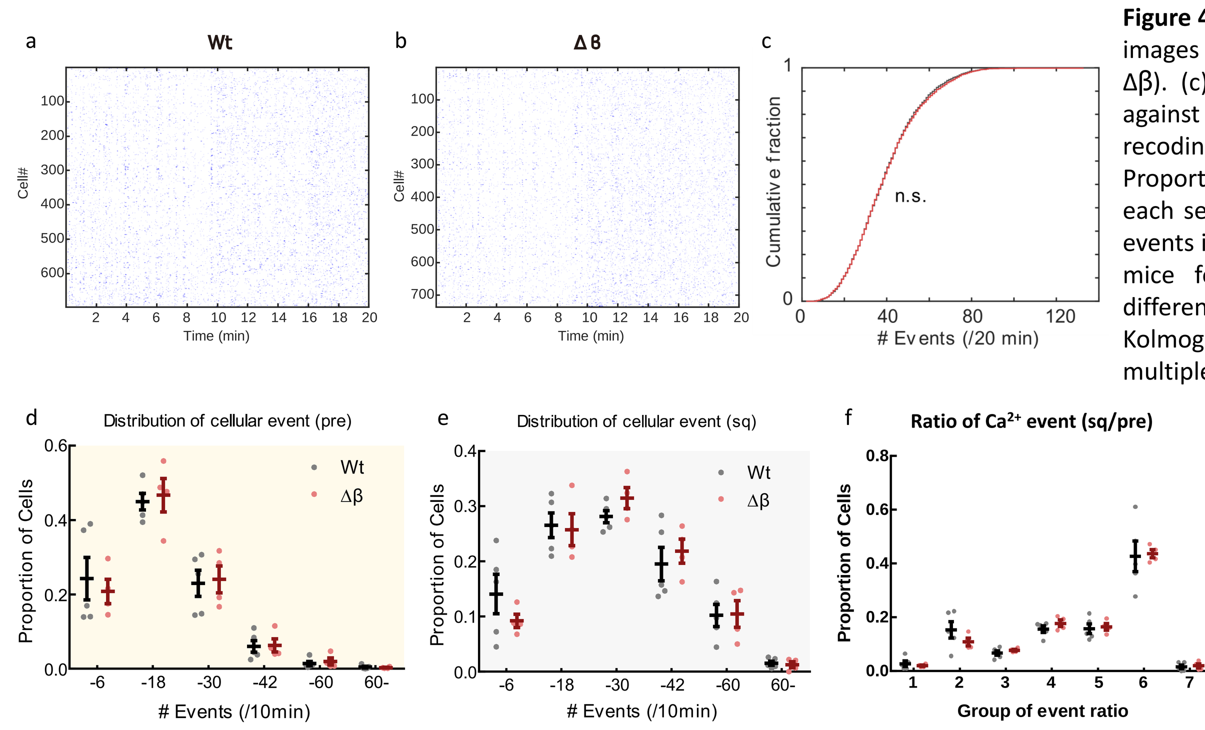


Figure 3. The activity of large ensembles were not so frequently. The number of activity event of extra large (XL, 20 or more cells; top right), large (L, 15-19 cells; top middle), medium (M, 10-14 cells; tope left), small (S, 5-9 cells; bottom right), several cells (SCs, 2-4 cells; bottm middle), single cell (SC; bottom left) in context session. n = 5 mice for Wt, n = 4 mice for Δβ. Mean ± S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$, (Adjusted *p*-value of Bonferroni's multiple comparison test).

Pcdhb-deletion did not affect cellular level activity



Group	Cellular activity	Ratio
1	Only pre	0
2	pre >> sq	0-.5
3	pre > sq	0.5-.75
4	pre = sq	0.75-1.25
5	pre < sq	1.25-2.0
6	pre << sq	2.0-
7	Only sq	∞

Figure 4. The cellular activity. (a, b) Representative images of raster plot of all recorded cells (a: Wt, b: Δβ). (c) Cumulative curve of proportion of cells against the number of active events during whole recoding session (pre 10 min + sq 10 min). (d, e) Proportion of cells on the number of Ca^{2+} events in each session. (f) The ratio of the number of Ca^{2+} events in sq to pre session. n = 5 mice for Wt, n = 4 mice for Δβ, Mean ± S.E.M. No significant difference was observed in all figures (c: Kolmogorov-Smirnov test, d-f: Bonferroni's multiple comparison test).

Pcdhb-deletion decreased the discrimination of contextual fear conditioning

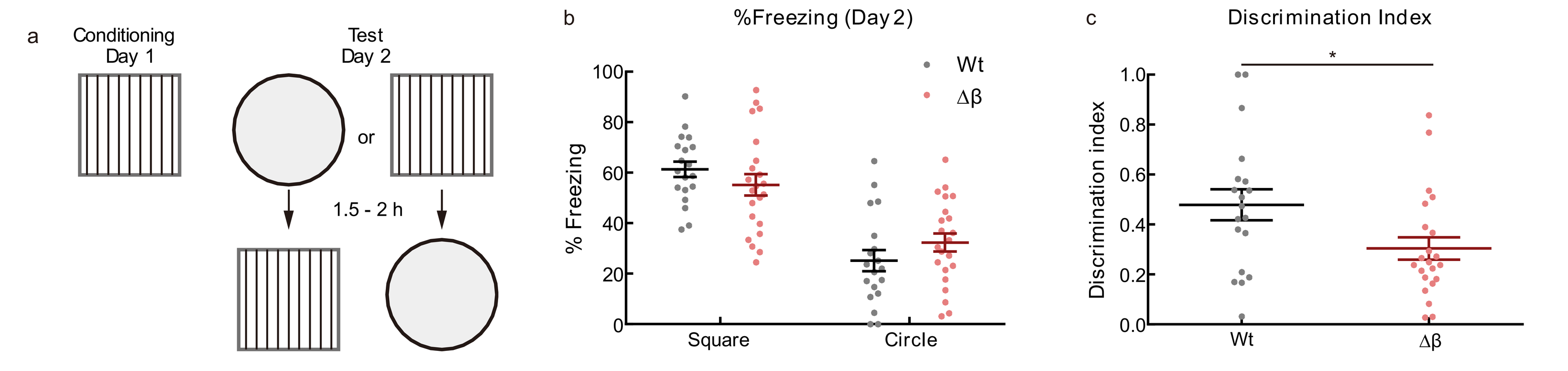


Figure 5. The contextual fear discrimination. (A) Schematic diagrams of experiment. (B) Freezing level in test session. (C) Discrimination index in test session. n = 19-22 mice in each group. Mean ± S.E.M. $^{*}p < 0.05$ (Unpaired t-test).

Discussion & Conclusion

➢ ***Summary of Results & Discussion***

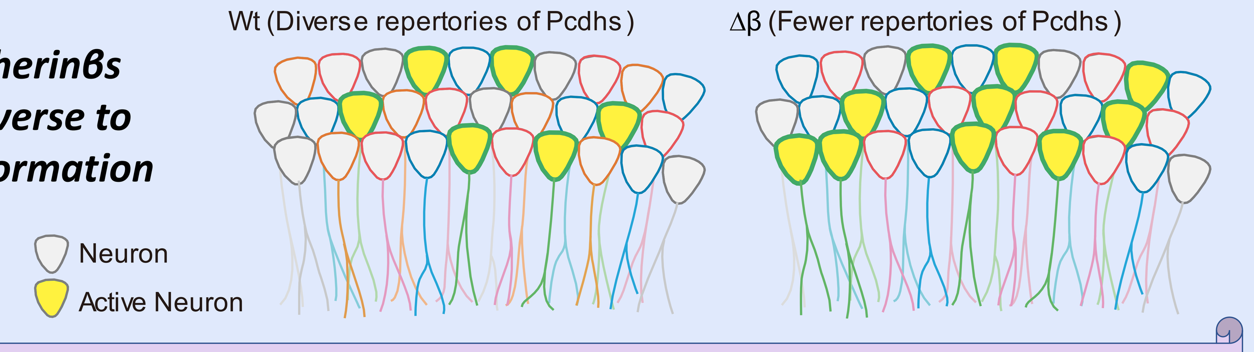
Fig. 2, 3 **Large Ensemble** — Increase, Rare activity
Fig. 1 **Repetitive activity** — Reduction
Fig. 4 **Cellular activity** — Similar
Fig. 2 **Cell sharing** — Increase
Fig. 5 **Contextual discrimination** — Impair

Clustered protocadherinβs do not affect the activity on the cellular level, but they are involved in population/ensemble activity and forming ensembles, which are sets of cells activated synchronously.

Increase the sharing cells between ensembles may impair contextual fear discrimination.

➢ ***Schematic diagrams of expected function***

Clustered protocadherinβs make ensembles diverse to avoid confusing information



Conclusion
Clustered protocadherinβs tune ensemble activity in the hippocampus and context discrimination

Acknowledgements

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We have no COI (Conflict of Interest) with regard to this presentation.