Artificial association of pre-stored information to generate a qualitatively new memory

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Summary

Memory is assumed to be stored in the brain as a cellular ensemble consisting of a set of neurons that is activated during learning. Although optical stimulation of a cellular ensemble is known to trigger the retrieval of the corresponding memory, it is unclear how the association of distinct information occurs at the cell ensemble level. Here, we show in mice that activation of a cell ensemble corresponding to two distinct memory events generates an artificial association between initially non-related events. In the context pre-exposure and immediate shock (IS) paradigm, mice failed to associate the shock with the pre-exposed context when the IS was delivered to their foot in a different context. Cells activated during the context pre-exposure and the IS in hippocampal CA1 and the basolateral amygdala (BLA) were targeted with channelrhodopsin-2, a light-activated cation channel. These cells were later simultaneously activated by optical stimulation in the mice's home cage. The next day, these mice exhibited freezing behaviour, an indicator of a fear response, in the pre-exposed context that was not originally associated with the shock. This artificial association shared characteristics with physiologically associated memories, such as N-methyl-D-aspartate receptor activity- and protein synthesis-dependence. Thus, the artificial activation of distinct cell ensembles, without any sensory input, is capable of generating an artificially associated memory. Furthermore, our finding suggests that the association of distinct units of information is achieved through the synchronous activity of distinct cell ensembles. This mechanism may underlie memory update by incorporating novel information into pre-existing networks to form qualitatively new memories.

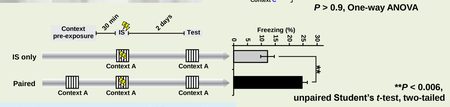
Results

① Cell assemblies corresponding to the pre-exposed context and the immediate shock (IS) in CA1 and the BLA

• Contexts A-C have the same impact on freezing levels at the first exposure

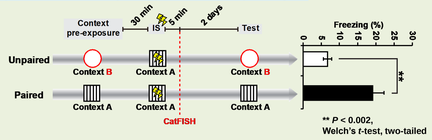
P > 0.9, One-way ANOVA

• Context pre-exposure facilitates effect of the IS



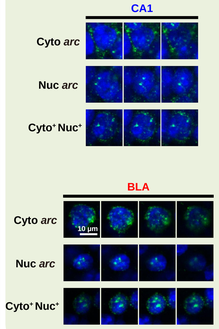
***P** < 0.006, unpaired Student's *t*-test, two-tailed

• Paired and Unpaired for context pre-exposure and IS paradigm

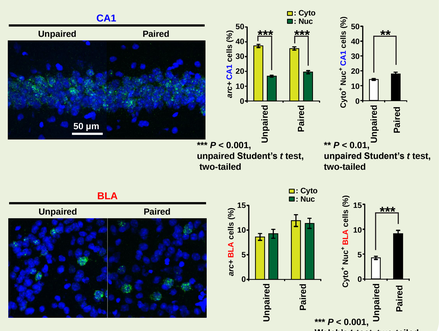


** *P* < 0.002, Welch's *t*-test, two-tailed

• Different cell staining profiles detected by *arc* catFISH



• *Arc* catFISH in Unpired and Paired conditions



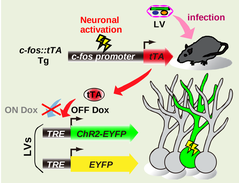
*** *P* < 0.001, unpaired Student's *t* test, two-tailed

** *P* < 0.01, unpaired Student's *t* test, two-tailed

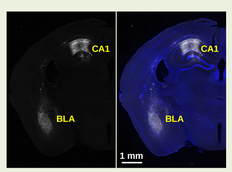
*** *P* < 0.001, Welch's *t*-test, two-tailed

② A system for the simultaneous targeting and activation of cell assemblies in CA1 and the BLA

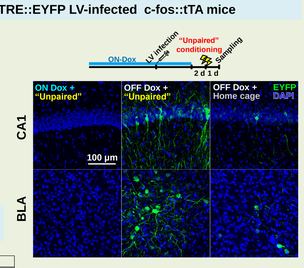
• Activity-dependent targeting of neurons in c-fos::tTA mice



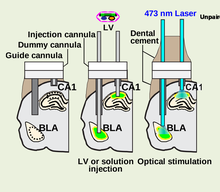
• Representative labeling pattern of CA1 and BLA cells in c-fos::tTA mice



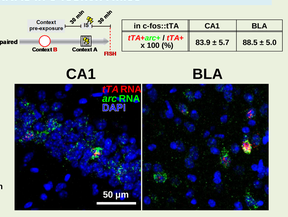
• Activity and OFF Dox-dependent expression of EYFP+ cells in CA1 and the BLA of TRE::EYFP LV-infected c-fos::tTA mice



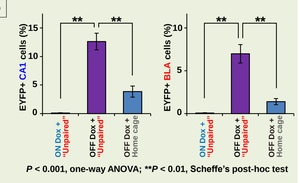
• Double-targeting of CA1 and the BLA



• Double-FISH to detect both of *tTA* and *arc* RNAs in c-fos::tTA mice



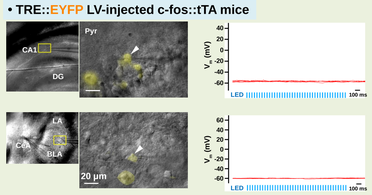
in c-fos::tTA	CA1	BLA
tTA+/*arc*+ / *tTA*+ x 100 (%)	83.9 ± 5.7	88.5 ± 5.0



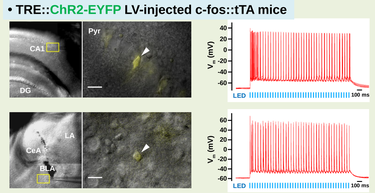
P < 0.001, one-way ANOVA; ***P* < 0.01, Scheffe's post-hoc test

• Blue LED light (20 Hz, 5 msec)-dependent spikes from the cells in CA1 and the BLA

• TRE::EYFP LV-injected c-fos::tTA mice

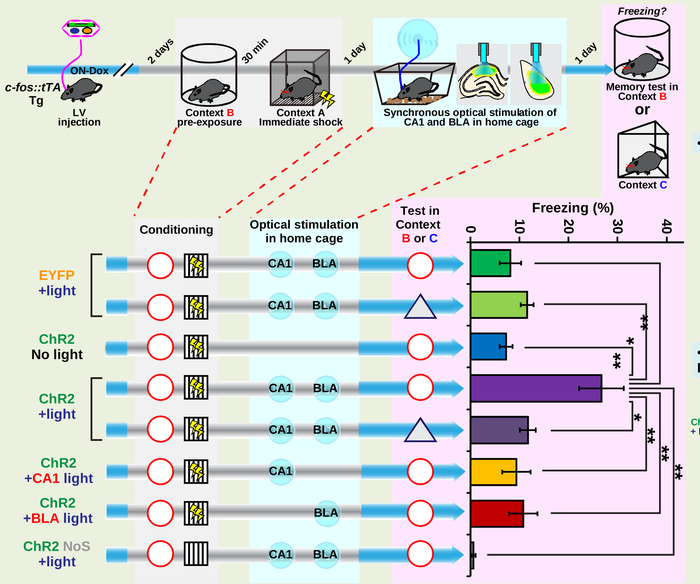


• TRE::ChR2-EYFP LV-injected c-fos::tTA mice



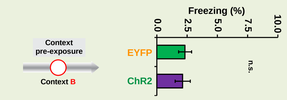
③ Synchronous activation of distinct cell ensembles creates an artificial association of corresponding information

• Optical stimulation (20 Hz, 10 msec, 2 min) of cell assemblies in CA1 and the BLA generates artificial association



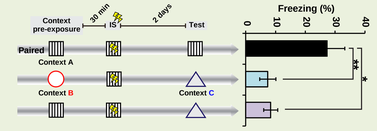
P < 0.001, one-way ANOVA; **P* < 0.05, ***P* < 0.01, Scheffe's post-hoc test

• Freezing responses in TRE::EYFP LV- and TRE::ChR2-EYFP LV-injected c-fos::tTA mice in context B at the first exposure



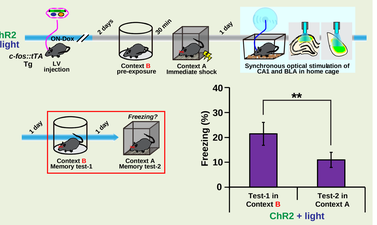
P > 0.8, Welch's *t*-test, two-tailed

• Control experiments in naive mice



P < 0.004, one-way ANOVA; **P* < 0.05, ***P* < 0.01, Scheffe's post-hoc test

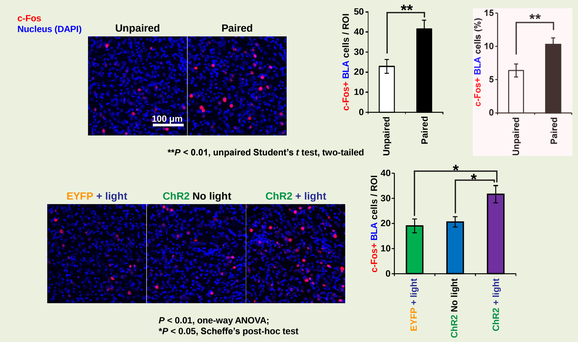
• ChR2 +light group show specific fear memory, linking the original context infomation



** *P* < 0.02, paired *t* test, two-tailed

④ Both the artificial and physiological association increase the representation of the sensory input in the BLA circuit

• Endogenous c-Fos expression 90 min after the test session in the BLA

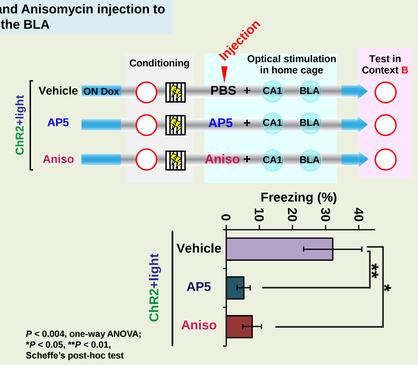


***P* < 0.01, unpaired Student's *t* test, two-tailed

P < 0.01, one-way ANOVA; **P* < 0.05, Scheffe's post-hoc test

⑤ Artificial association requires NMDAR activity and *de novo* protein synthesis during optical stimulation

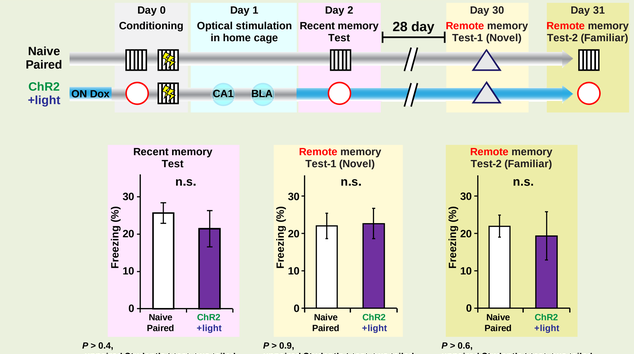
• D-AP5 and Anisomycin injection to CA1 and the BLA



P < 0.004, one-way ANOVA; **P* < 0.05, ***P* < 0.01, Scheffe's post-hoc test

⑥ Temporal changes in artificial associative memory and physiological associative memory are comparable

• Recent and Remote memory test of artificial and physiological associative memory

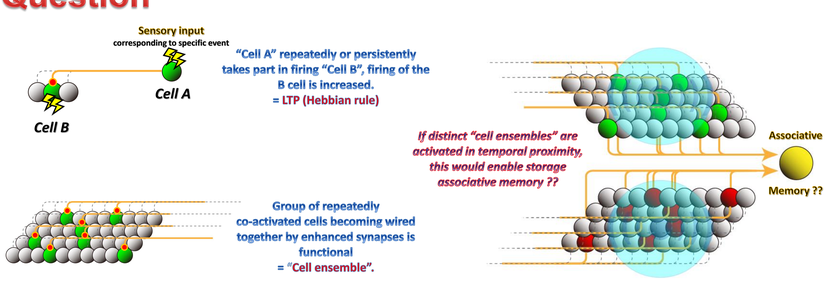


P > 0.4, unpaired Student's t-test, two tailed

P > 0.9, unpaired Student's t-test, two tailed

P > 0.6, unpaired Student's t-test, two tailed

Question



Methods

➢ **Virus injection**

The pLenti-TRE::EYFP and pLenti-TRE::ChR2-EYFP plasmids were constructed and used for LV preparation as described previously (Goshen, I. et al., (2011) *Cell* 147, p678-). The LVs were stereotaxically injected into CA1 and the BLA of the right hemisphere of c-fos::tTA mice purchased from the MMRRC.

➢**Ex vivo electrophysiology**

Transverse brain slices were prepared from, and the membrane potential of ChR2-EYFP-expressing neurons in the CA1 and BLA regions was recorded under whole-cell current-clamp mode. Light stimulation (465 nm, 20 Hz, 5 msec, 40-pulse) was delivered with a high-power LED illumination system.

➢**Histology**

An *arc* catFISH analysis was performed according to previous studies (Guzowski, J. F. et al., (1999) *Nat. Neurosci. 2*, p1120-). For immunohistochemistry, brain sections were incubated with anti-GFP (1:1000, Molecular Probes, A11122) and/or anti-c-Fos (1:1000, Santa Cruz, sc-52-G) antibodies followed by treatments with secondary antibodies and then subjected to confocal microscopy.

➢**Behavioural analysis**

Mice were pre-exposed to context for 6 min, returned to the home cage for 30 min, and then given a 0.8 mA foot shock for 2 sec in context A after 5 sec of acclimation. The 'unpaired' paradigm used distinct contexts for pre-exposure (context B) and the IS in context A. The 'paired' paradigm used the same context (context A) for pre-exposure and the IS. The memory tests of the 'unpaired' and 'paired' conditions were performed in contexts B and A, respectively. Cannula-implanted and LV-injected c-fos::tTA mice were maintained on Dox food pellets (40 mg/kg). The mice were taken OFF Dox for 2 days and then trained with the 'unpaired' paradigm. After 24 h, the mice were anaesthetised for ~4 min and optic fibres were attached through the cannula. The mice were returned to the home cage for 20-25 min before optical stimulation (473 nm light, 20 Hz, 10 msec) was delivered in the home cage for 2 min. Five minutes after the end of the stimulation, the optic fibre was detached, and the mice were returned to the home cage. The mice were put into context B for the fear memory test.

Conclusions

- ***The arc catFISH revealed expression of neuronal assemblies that were activated during the context pre-exposure and the IS in hippocampal CA1 and the BLA.***
- ***The entirely artificial activation of the two separate sets of neuronal assemblies, without any new sensory input from the conditioned and unconditioned stimuli (the context pre-exposure and foot shock), was capable of connecting two independent events.***
- ***This artificially associated memory shared several characteristics with a physiologically associated memory: Both depended on NMDAR activity and protein synthesis, both were long lasting (at least four weeks), and both underwent generalization.***
- ***This result suggests that synchronous activation of cell assemblies corresponding to two distinct events could generate an artificial link between these memory episodes.***
- ***This mechanism may underlie memory updating by incorporating novel information into pre-existing networks to form qualitatively new memories.***