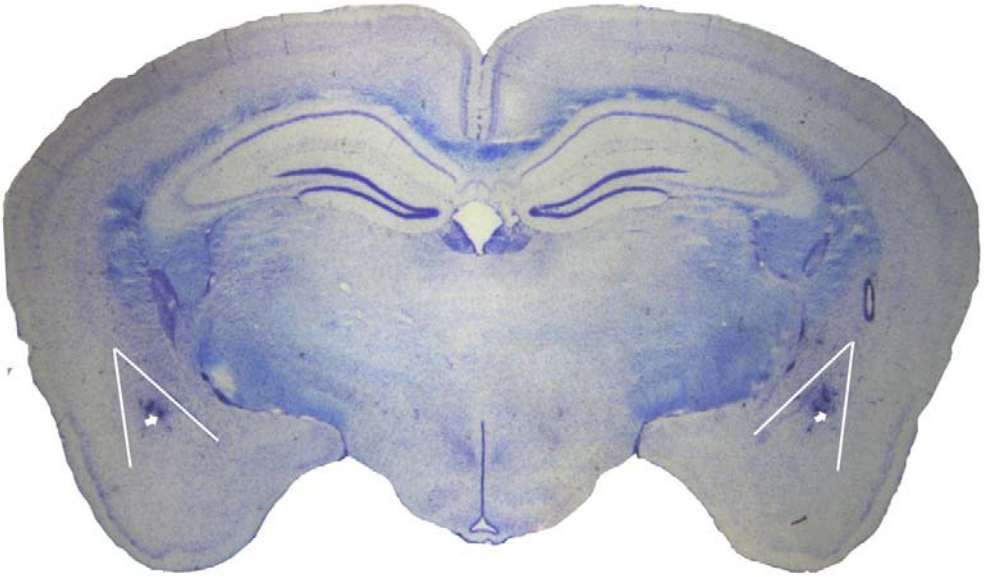
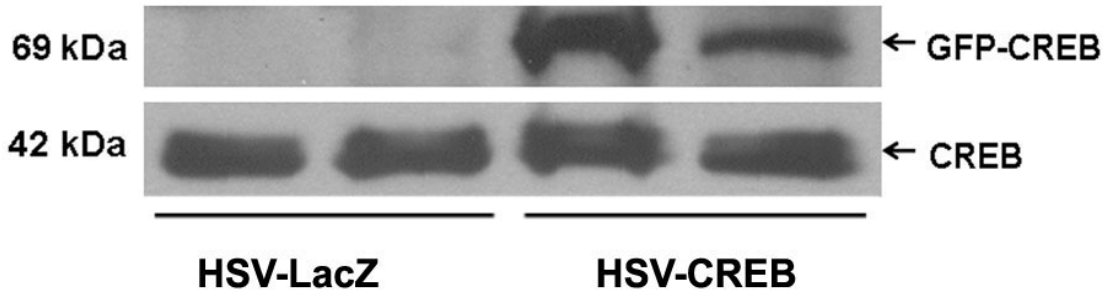
CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala

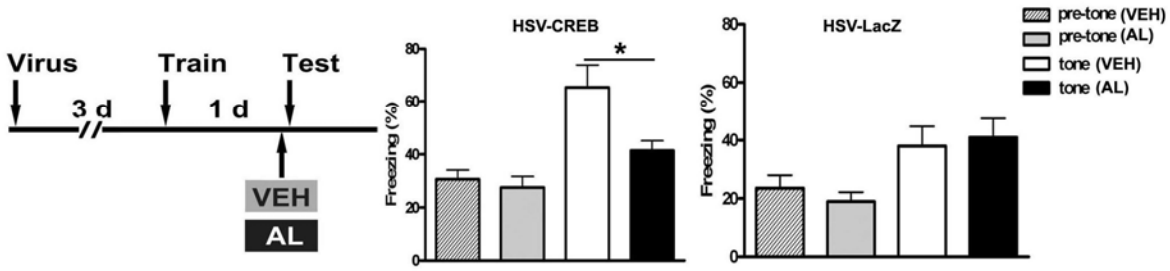
Yu Zhou[1,2,3,4], Jaejoon Won[1,2,3,4], Mikael Guzman Karlsson[1,2,3,4], Miou Zhou[1,2,3,4], Thomas Rogerson[1,2,3,4], Balaji J.[1,2,3,4], Rachael Neve[5], Panayiota Poirazi[6], Alcino J. Silva[1,2,3,4,*]



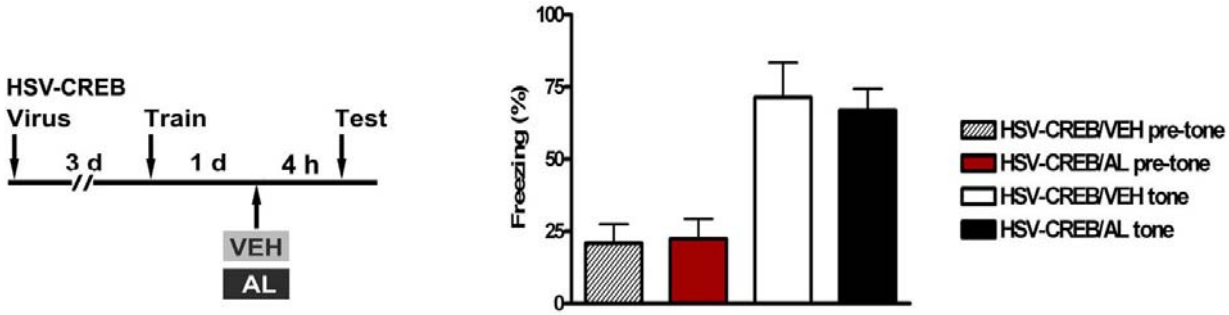
Supplementary Fig.1 Illustration of cannula tip positions in amygdala (white arrow). Crystal violet staining.



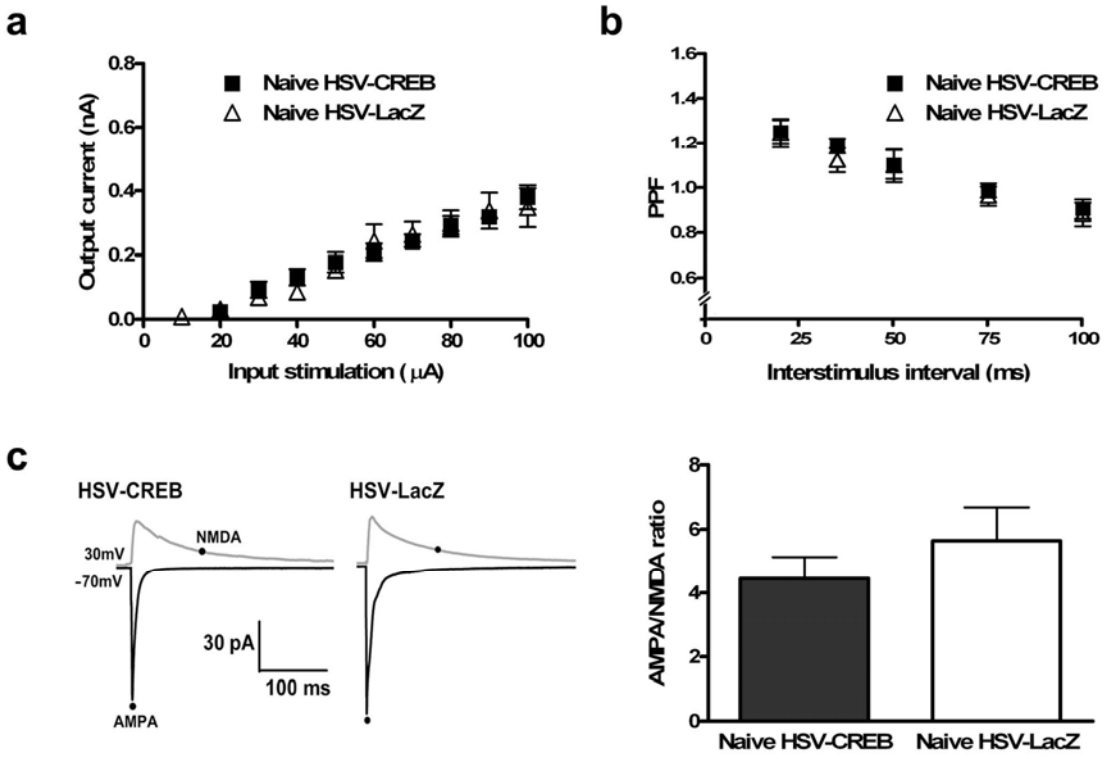
Supplementary Fig.2 Representative western blot analysis showed that HSV-CREB viral infection increased total CREB levels in the amygdala. Endogenous CREB (42 kDa) and viral GFP-CREB (69 KDa) were measured in amygdala tissue punches from mice transfected with either HSV-CREB-AlstR or HSV-LacZ-AlstR. As expected, mice transfected with HSV-LacZ had no detectable GFP-CREB.



Supplementary Fig.3 Inactivation of the subpopulation of LA neurons transfected with HSV-CREB-AlstR disrupted long-term memory (LTM) for tone conditioning. Left: schematic of the experimental design. Middle and Right: histogram showing that AL during test (24 h after conditioning) blocks freezing in HSV-CREB-AlstR mice (middle), but has no effect on HSV-LacZ-AlstR mice (right). Pre-tone (VEH) or AL refers to baseline freezing (before tone presentation) of VEH or AL treatment group, tone (VEH) or AL refers to test freezing (during tone presentation) of VEH or AL group. Mice received HSV-CREB-AlstR (or HSV-LacZ-AlstR) vector and were tested after AL (or VEH) administration. n = 12 for each HSV-CREB group and n=10 for each HSV-LacZ group. *P < 0.05 as indicated.



Supplementary Fig.4 AL infusion 4 h before testing had no effect on retrieval. Left: schematic of the experimental design. AL or vehicle was injected into the LA 4 h (instead of 30 min) before testing tone conditioning. Right: Histogram showing that AL infusion 4 h before testing had no effect on retrieval. *n* = 8 to 10 animals for each group.



Supplementary Fig.5 HSV-CREB vector transfection did not affect the evoked basal synaptic transmission or PPF of LA neurons in naïve mice. (**a**) Input-output curve showing that HSV-CREB does not change the basal synaptic transmission in LA neurons of naïve mice. The intensity of extracellular stimulation is varied from 10 μm to 100 μm. (**b**) Averaged data show that there is no difference between the PPF ratios of HSV-CREB and HSV-LacZ transfected cells of naïve mice. (**c**) HSV-CREB does not affect AMPA/NMDA EPSC ratios of transfected LA neurons. Left: sample AMPA/NMDA currents induced by electrical stimulation of the thalamic pathway in HSV-CREB and HSV-LacZ neurons. Right: averaged data showing that there is no difference on AMPA/NMDA EPSC ratios of HSV-CREB (*n* = 14 from 5 mice) and HSV-LacZ neurons (*n* = 12 from 5 mice).

Supplementary table: Membrane properties of LA pyramidal neurons after HSV-CREB virus infection *in vivo*

	HSV-CREB+ (n=58)	HSV-CREB– (n=40)	HSV-LacZ+ (n=12)	HSV-LacZ– (n=12)
RMP (mV)	–58.9 ± 0.7	–60.4 ± 0.6	–59.2 ± 1.3	–61.1 ± 1.5
[a]Spike threshold (mV)	–38.8 ± 0.9*	–35.6 ± 1.0	–35.4 ± 1.0	–35.7 ± 1.0
[a]Spike amplitude (mV)	91.8 ± 2.0	94.6 ± 2.8	93.5 ± 3.0	95.1 ± 3.5
[a]Spike half-width (ms)	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
Input resistance (MΩ)	151.3 ± 9.6	138.3 ± 8.6	140.7 ± 10.4	136.9 ± 10.8

Results are mean ± SEM. *$P < 0.05$ as indicated.

[a] First spike from each trace that showed the smallest number of evoked spikes (usually only 1 spike) in response to small depolarizing step (600 ms with the intensity of either 50,100 or 150 pA).