

SUPPLEMENTAL ONLINE MATERIAL

Silent synapse-mediated reorganization of the glutamatergic projection from amygdala to nucleus accumbens is necessary for incubation of cocaine craving

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Supplementary Figures

Self-administration for rats used in the different experiments

Rats with a history of saline or cocaine self-administration were used to examine generation and maturation of silent synapses in the BLA-to-NAc glutamatergic projections (**Figs. 1-6** in main text). The performance of the rats during self-administration training is summarized in **Fig. S1**.

Additional evidence for re-silencing of silent synapses by inhibition of CP-AMPARs

In rats that were trained to self-administer cocaine and underwent 45 withdrawal days, we performed the minimal stimulation assay within the BLA-to-NAc projection to estimate the level of silent synapses before and during perfusion of the CP-AMPA-selective antagonist Naspam. We observed that inhibition of CP-AMPA caused the re-emergence of silent synapses in this projection in cocaine-experienced rats (**Fig. 5** in main text). For this effect of cocaine, saline-experienced rats were used, in which no such an effect was observed (statistics reported in the main text). Here, we provide a summary figure (**Figs. S2**) showing these control results.

Additional data for LTD-induced reversal of incubation of cocaine craving

We demonstrated in the main text that induction of in vivo LTD on withdrawal day 45 reversed the incubation of cocaine craving (**Fig. 7**). Here, we provide an additional figure from individual rats to compare their cocaine seeking behaviors on withdrawal days 1 and 45. In sham controls, most rats exhibited increased cocaine seeking on withdrawal day 45, whereas rats that received LTD induction in the BLA-to-NAc projection, cocaine seeking did not show significantly different cocaine seeking between withdrawal days 1 and 45 (**Fig. S3**).

Supplementary figure legends

Figure S1. Summary of saline or cocaine self-administration training data for experiments presented in the main text. (A) Summarized training data for rats used in Fig. 2C-G. (B) Summarized training data for rats used in Fig. 3A-D. (C) Summarized training data for saline or cocaine for rats used in Fig. 3E-I. (D) Summarized training data for rats used in Fig. 4. (E) Summarized training data for rats used in Fig. 5-6.

Figure S2. Additional summary for re-silencing of previously silent synapses by inhibition of CP-AMPA receptors on withdrawal day 45. EPSCs elicited by minimal stimulations (recorded at -70 and +50 mV) from BLA-to-NAc synapses 45 days after saline self-administration are shown. A-C Example (A) and trials (B, C) of EPSCs from a saline-withdrawn rat before and during perfusion of Naspm. (D) Summarized results showing that, on withdrawal day 45 day from cocaine self-administration, application of Naspm did not affect the failure rate of EPSCs at either -70 or +50 mV at BLA-to-NAc excitatory synapses. (E) A summary showing that perfusion of Naspm did not affect the level of silent synapses in the BLA-to-NAc shell afferent in saline-experienced rats. This is a control result for Figure 5 in the main text.

Figure S3. Additional figure for in vivo LTD-induced reversal of incubation of cocaine craving. Summarized results showing that cocaine seeking was significantly higher on withdrawal day 45 than on day 1 in sham control rats (left), whereas induction of LTD in the BLA-to-NAc glutamatergic projection reversed the time-dependent increase of cocaine seeking (incubation of craving) (right).

Fig. S1

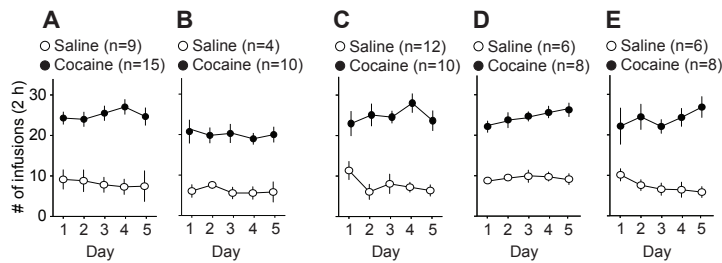


Fig. S2

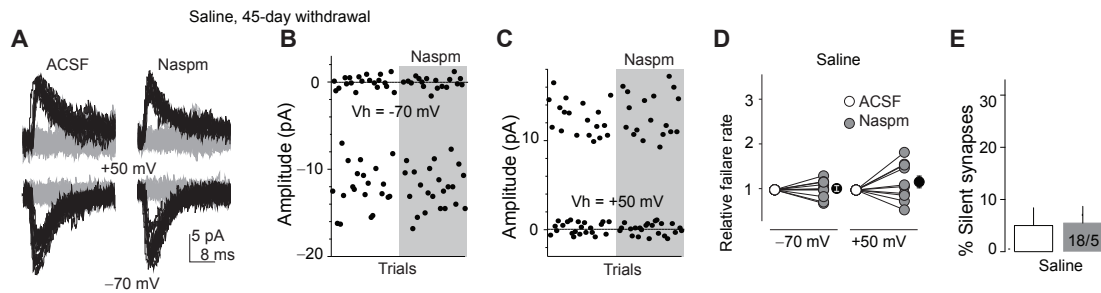


Fig. S3

