Experiment 1: contribution of nmdar subunits in formation of fix-c and esc-c memo...

To determine whether NMDAR subunits were differentially regulated in miceconditioned by Fix-C and Esc-C (Itzhak & Anderson, 2012) we performed quantitativereal-time polymerase chain reaction (qPCR) and immunoblot analyses (n= 3-4mice/group). Bilateral hippocampus was dissected 24h after conditioning andsubsequently analyzed. In the hippocampus NR2A and NR2B are the predominant NR2subunits that comprise the NMDAR (Monyer et al., 1994). The NMDAR subunits NR1, NR2A and NR2B are encoded by the genes Grin1, Grin2a and Grin2b, respectively. Wefocused on the hippocampus because of its role in spatial/contextual memory.

Experimental groups for Fix-C and Esc-C included the following: Coc-Paired: saline wasgiven in one compartment at mornings and cocaine in the other compartment 3h later. Coc-Unpaired: saline was given in one compartment at mornings and 3h later mice were reexposedto the conditioning apparatus in the absence of drug; cocaine was administered(30min later) in the home cage. Saline-Paired: saline given in both compartments. ForqPCR the saline, paired and unpaired (Fix-C and Esc-C) groups were analyzed whereas forwestern blot analyses, only the saline controls and paired groups of Fix-C and Esc-C wereanalyzed.

qPCRTwenty-four hours following conditioning sessions, mice were tested for CPP andwere sacrificed 20 min later. Bi-lateral hippocampus was dissected and stored in RNAlater(Qiagen). Equal amounts of total RNA were reverse-transcribed and subjected to qPCRanalysis (n= 4 mice/group). Custom designed qPCR arrays (Qiagen) were used to evaluatechanges in gene expression profiles in response to different experimental conditions.

Cycle threshold (Ct) values were used to compare differences in expression levels amongthe groups. Since saline groups from both Fix-C and Esc-C experiments received the sametreatment and there was no significant difference between them, they were combined toserve as control.