Experiment 3: reconsolidation of fix-c and esc-c memory

Administration of MK-801 before the CPP test (pre memory retrieval) preventeda reduction in place preference for Fix-C [F(1, 48)= 8. 904; p= 0. 004; group effect] but it hadno effect on Esc-C (Fig 2. 3B, C). For Fix-C, there was a significant time effect[F(2, 48)= 48. 437; p <0.

001] and a significant group x time interaction [F(2, 48)= 3. 246; p= 0. 048]. For Esc-C, there was a significant time effect [F(2, 33)= 85.

508; p <0. 001] but nosignificant group x time interaction (p> 0. 05). The results suggest that the first exposure tothe CPP context may elicit extinction in the Fix-C but not Esc-C group and MK-801inhibited the extinction in the Fix-C group.

In the next experiment MK-801 was administered 6 min following the first CPPtest (post memory retrieval). A dose of 0. 1mg/kg MK-801 attenuated subsequentexpression of Fix-C. Two-way ANOVA showed a significant time effect [F(2, 39)= 43.

151; p <0. 001] but no significant group effect nor group x time interaction (p> 0. 05). Post hocanalysis showed that on day 12, MK-801-treated animals showed reduced CPP comparedto saline-treated mice (p= 0. 012), suggesting that MK-801 disrupted memoryreconsolidation.

With respect to Esc-C, two-way ANOVA comparing the dose effect ofMK-801 on disruption of reconsolidation showed a significant group (MK-801 dose)effect [F(2, 66)= 5. 747; p= 0. 005], a significant time effect [F(2, 66)= 83. 735; p <0.

001] but nosignificant group x time interaction. Results suggest that while a low dose (0. 1mg/kg)MK-801 was sufficient to disrupt Fix-C memory reconsolidation, a higher dose of MK-801 (0. 3mg/kg) was required for disruption of Esc-C memory reconsolidation (Fig.

2. 3E; day 12). In all subsequent reconsolidation experiments drugs were administered followinga 6 min CPP test (post memory retrieval). For both Fix-C and Esc-C, the 6 min exposureto the context was sufficient to express the acquisition of CPP (Pre-CPP vs Retrieval; p <0. 001; Fig 2.

4). Ifenprodil (group effect) significantly disrupted reconsolidation ofboth Fix-C and Esc-C memory [for Fix-C: F(1, 48)= 4. 123; p= 0. 048; for Esc-C: F(1, 39)= 11.

832; p= 0. 001; Fig 4B, C]. There was a significant time effect [for Fix-C: F(2, 48)= 31.

171; p <0. 001; for Esc-C: F(2, 39)= 25. 697; p <0. 001] but no significant group xtime interaction. Administration of ifenprodil either in the home cage in the absence ofmemory retrieval or 6h after memory retrieval had no effect on Esc-C CPP (Fig 4D), suggesting that ifenprodil disrupted memory reconsolidation. To further validate theinvolvement of NR2B in Fix-C and Esc-C memory reconsolidation, the effects oftraxoprodil was investigated. Traxoprodil is another NR2B receptor antagonist which haspreviously been tested in clinical trials for the treatment of depression (Preskorn et al., 2008), dyskinesia and Parkinsonism (Nutt et al.

, 2008). Administration of traxoprodilfollowing memory retrieval significantly reduced subsequent expression of CPP in bothFix-C and Esc-C (Fig 2. 4E, F). Two-way ANOVA showed a significant group effect forEsc-C [F(1, 42)= 9. 466; p= 0.

004] but not Fix-C [F(1, 42)= 2. 555; p= 0. 117]. There was asignificant time effect for both Esc-C [F(2, 42)= 75. 191; p <0.

001] and Fix-C [F(2, 42)= 35. 280; p <0. 001] but no significant group x time interaction in either case. Post hoc analysisshowed that traxoprodil-treated mice displayed significantly lower CPP scores thanvehicle-treated mice for both Esc-C (p <0.

001) and Fix-C (p= 0. 005) on day 12. We then sought to isolate the contribution of different signaling moleculesdownstream of the NMDAR in cocaine-memory reconsolidation. Post-retrievaladministration of the nNOS inhibitor 7-NI reduced the magnitude of subsequent CPP thatwas acquired by Fix-C but not Esc-C schedule (Fig. 2. 5B, C), suggesting disruption ofFix-C [F(1, 45)= 6.

558; p= 0. 014] but not Esc-C [F(1, 48)= 0. 0166; p= 0. 898] memoryreconsolidation. There was a significant time effect for Fix-C [F(2, 45)= 42. 089; p <0. 001]and Esc-C [F(1, 48)= 29.

907; p <0. 001] but no significant group x time interaction. Thedisruption of Fix-C memory reconsolidation by 7-NI is consistent with our previousstudies (Itzhak & Anderson, 2007). We then investigated the involvement of ERK in reconsolidation of Fix-C andEsc-C memory. The ERK kinase (MEK) inhibitor

SL327 disrupted Esc-C but not Fix-Cmemory reconsolidation (Fig 2. 5D, E). Two-way ANOVA showed no significant groupeffect for Esc-C [F(1, 36)= 3. 904; p= 0.

056] nor Fix-C [F(1, 51)= 0. 873; p= 0. 354]. However, there was a significant time effect [for Esc-C: F(2, 36)= 49.

884; p <0. 001; for Fix-C: F(2, 51)= 63. 774; p <0. 001] and a significant group x time interaction for Esc-C[F(2, 36)= 3. 727; p= 0. 034] but not for Fix-C [F(2, 51)= 0. 344; p= 0.

71]. Post hoc analysis founda significant reduction in CPP on day 12 for Esc-C (p= 0. 014) but not Fix-C (p> 0. 05). Taken together, these results suggest that Fix-C memory engages NO signaling whileEsc-C memory may bypass the dependence on NO and engages the NMDAR-dependentERK signaling pathway.