

# [Materials and methods (conditioning procedure)](https://assignbuster.com/materials-and-methods-conditioning-procedure/)

SubjectsMale C57BL/6J mice (8 weeks old) were purchased from Jackson Laboratories(Bar Harbor, Maine). Mice were housed in groups of 5/cage with food and water adlibitumand were acclimatized to the vivarium for one week before experiments began. Animal care was in accordance with the Guide for the Care and Use of LaboratoryAnimals (National Research Council, National Academy Press, 1996) and was approvedby the University of Miami Animal Care and Use Committee. DrugsCocaine HCl and (+)-MK-801 hydrogen maleate (dizocilpine) were dissolved insaline (0.

9% NaCl). The NR2B antagonist ifenprodil [?-(4-Hydroxyphenyl)-?-methyl-4-benzyl-1-piperidineethanol(+)-tartrate salt] was dissolved in distilled water. Traxoprodil[(1S, 2S)-1-(4-hydroxy-phenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol], anotherNR2B-containing NMDAR antagonist (Chenard et al., 1995), and the nNOS inhibitor 7-nitroindazole (7-NI) were dissolved in a 1: 3: 6 mixture of DMSO, polyethylene glycoland distilled water, respectively. The MEK inhibitor SL327 was dissolved in 40% DMSO(Atkins et al., 1998). All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Drugs and vehicles were administered intraperitoneally (i. p.) in a volume of0. 1ml/10g. Conditioning procedurePlace preference was monitored using custom-designed Plexiglas cages (Opto-Max Activity Meter v2.

16; Columbus Instruments) as previously described (Liddie et al., 2012). The training context consisted of two compartments. One compartment had blackand white striped walls and a white floor covered with stainless steel grid; the othercompartment had black walls and smooth black floor. Each compartment was scanned by7 infrared beams. A null zone 8 cm wide was assigned at the interface of the twocompartments to ensure that only full entry into each compartment is registered as ‘ real’time spent in each zone. On the first day, mice were habituated (15min) to the trainingcontext; preconditioning compartment-preference/aversion was determined.

Preconditioningaverage times spent in the black, striped and null zones during 1200 secondswere 456±12, 516±13 and 217±9 seconds, respectively. Half the subjects showed slightpreference for one side or the other. Accordingly, mice were paired with cocaine in theless preferred compartment. Although this may be viewed as a biased design, half of themice were paired with cocaine in the black compartment and the other half were pairedwith cocaine in the black and white-striped compartment making this design ‘ partiallybiased’. Following habituation, mice were conditioned over 4 days by a) 11.

25mg/kg(Fix-C) or b) 3, 6, 12 and 24mg/kg; one dose per day (Esc-C) as we previously described(Itzhak & Anderson, 2012). Doses were chosen to control for total amount of cocaineadministered over 4 days. Post-conditioning average time spent in the null zone during1200 seconds CPP test was 192±7 seconds. Likewise time spent in the null zonefollowing pharmacological treatments, pre- and post-CPP, was not significantly differentthan vehicle treatment (201+9 seconds).