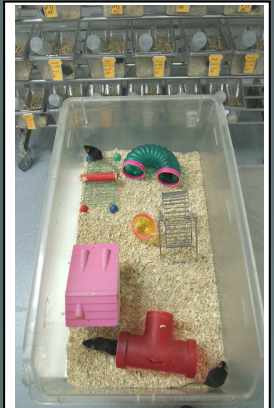


Abstract

Environmental manipulations during adolescence, a critical period of development, can have important consequences on subsequent vulnerability to drugs of abuse. Whereas many studies have demonstrated that negative environmental conditions increase vulnerability to drugs, little is known on whether positive environmental conditions such as enriched environments (EE) have protective effects against addiction. EE are known to enhance sensory, cognitive and motor stimulation in addition to increase learning and memory, facilitate recovery from brain injuries and to reduce the insurgence of neurodegenerative disorders. Our EE set-up consisted of housing cages that were bigger than standard cages and contained constantly a running wheel and a small house and four-five toys that were changed once a week with new toys of different shape and color (1).

We have previously shown that mice exposed to EE from weaning to adulthood show a decrease in cocaine-induced locomotor activity (2). More recently we found that the rewarding effects of cocaine as well as its ability to induce behavioral sensitization are reduced in mice reared in EE compared to standard environments (SE) controls (3). These effects were not paralleled by changes in cocaine-induced elevations of dopamine levels but were rather associated to reduced ability of cocaine to induce expression of immediate early genes such as zif-268 in the nucleus accumbens.

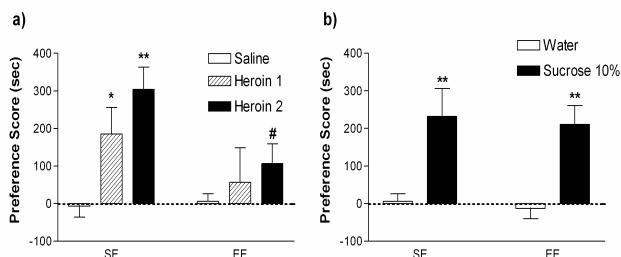
Here, we aimed at investigating whether early exposure enriched environment could also reduce the effects of heroin. We used conditioned place preferences paradigms to study the rewarding effects and behavioral sensitization to study the long term behavioral adaptations associated with repeated administration of heroin. We also investigated heroin-induced elevation of accumbal dopamine by *in vivo* microdialysis and expression of immediate early genes to understand the mechanisms involved in the influences of environmental enrichment on heroin addiction.



Setup for Environmental Enrichment

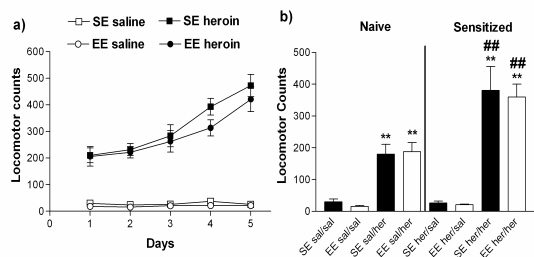
Results

1. Conditioned Place Preferences



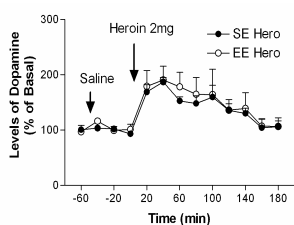
EE mice are less sensitive than SE mice to the reinforcing effects of heroin but not of food

2. Behavioral Sensitization



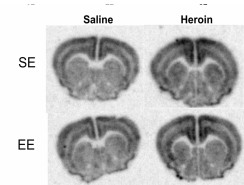
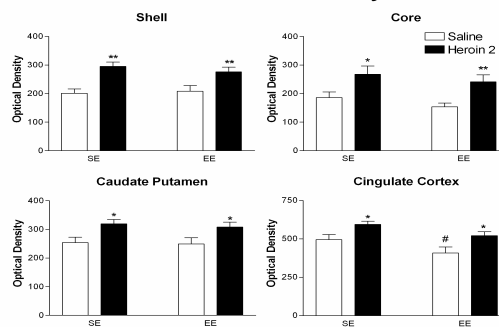
The activating and sensitizing effects of heroin do not differ between EE and SE mice

3. *In vivo* Microdialysis



Heroin-induced increases in extra-cellular dopamine in the nucleus accumbens does not differ between EE and SE mice

4. *In situ* Hybridization for zif-268



Heroin-induced increases in zif-268 expression in striatal areas do not differ between EE and SE mice.

Basal levels of zif-268 expression in the cingulate cortex are significantly lower in EE compared to SE mice

Methods

• Inbred C57Bl/6 mice were housed together in groups of 4, after weaning (3 weeks of age) for two months either in a standard environment (SE) or an enriched environment (EE). The SE consisted of common cage housing (30x15x15 cm). The EE constituted a larger (75x45x25 cm) cage containing constantly six-seven toys, which included a wheel and a small house that were randomly changed once a week. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for the care of laboratory animals.

• Conditioned place preference (CPP) paradigm consisted of one pre-test session, eight (four heroin/sucrose + four saline/water) conditionings and one test session. CPP was measured as the difference in the time spent in the heroin-paired compartment before and after the conditionings.

• Behavioral sensitization to the locomotor-activating effects of heroin consisted of two phases: development and expression. For the development of behavioral sensitization, five injections of heroin (1 mg/kg s.c.) or saline were administered every other day. Expression of behavioral sensitization was measured 30 days after the last injection.

• *In vivo* microdialysis experiments were performed in freely-moving mice after probe implant in the nucleus accumbens (3). Dopamine samples were collected every 20 min and analyzed by HPLC coupled to electrochemical detection. When dopamine levels were stable, mice received an injection of saline followed by an injection of heroin (2 mg/kg s.c.).

• *In Situ* Hybridization with [35 S] (UTP)-labelled RNA probe for zif-268 was performed on coronal brain sections (14 μm) (3). Sections were then exposed to phosphorimager screen for 7 days and quantitative analysis was performed following phosphorimager scan.

References

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- Beard E, Dovero S, Belin D, Duong S, Jackson-Lewis V, Przedborski S, Piazza P.V., Gross C.E., Jaber M. 2003 Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: involvement of dopamine transporter and trophic factors. *J Neurosci*. 23:10999-11007
- Solinas M., Thiriet N., El Rawas R., Lardeux V., and Jaber M. Environmental enrichment during early stages of life reduces the behavioral, neurochemical and molecular effects of cocaine. *Neuropsychopharmacology*. In press

Conclusions

- Heroin is less rewarding in EE compared to SE mice as measured by conditioned place preference. This suggests that environmental enrichment during adolescence has positive consequences not only against psychostimulant but also against opiate addiction.
- Natural rewards are equally rewarding in SE and EE mice indicating that environmental enrichment selectively reduces drug rewards.
- The activating effects of acute or repeated administration of heroin, heroin-induced increases in dopamine levels in the NAc and heroin-induced increases in expression of the immediate early gene zif-268 do not differ between EE and SE.
- Activating, but not rewarding effects of heroin, are paralleled by activation of the mesolimbic dopaminergic system indicating that rewarding effects of heroin can be independent from dopamine neurotransmission.
- The reduced expression of zif-268 in the cingulate cortex are consistent with the enhanced ability of EE mice to habituate to novelty.
- Altogether the present results provide support for the hypothesis that environmental enrichment during early ages may be a fundamental factor in reducing the vulnerability to heroin addiction.