Differential Morphine Withdrawal Profiles in High- and Low-Saccharin Preferring Rats

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Background
• Research with humans and rodents has found a positive relationship between excessive intake of sweetened dietary substances and the tendency to abuse drugs. Common neurobiological correlates between substance dependence and pathological behaviors such as overeating, gambling, and drug abuse have been suggested.2
• Drugs and natural rewards such as food, sex, and exercise increase the release of dopamine in the mesolimic system which includes the ventral striatum. Better knowledge of the common neural mechanisms underlying drug- and food-motivated behaviors can help us to effectively address these addictive behaviors clinically.3
• Two lines of selectively bred rats that preferentially consume high (HiS rats) and low (LoS rats) amounts of saccharin are presently used to study characteristic differences during acute morphine withdrawal between these phenotypes. Behavioral studies have already shown that HiS rats self-administer ethanol, heroin, and cocaine more readily than LoS rats.4
• These animal lines have been extensively used to model genetic predisposition to addictive behavior as they represent drug-prone and drug-resistant phenotypes.
• It has been suggested that LoS animals consume less drugs because they experience greater withdrawal symptoms, making a drug more aversive.5 We aim to test this hypothesis by investigating whether severity of the negative withdrawal syndrome from morphine is different in LoS and HiS animals. Moreover, we look to determine whether sex has an effect on withdrawal-induced anxiety among the HiS and LoS phenotypes.
• We measured the acoustic startle reflex following acute morphine exposure using a paradigm called withdrawal-potentiated startle (WPS). Increases in startle are indicative of anxiety and withdrawal.5,6
• We also used morphine conditioned place aversion (CPA), which measures the degree to which an animal avoids an environment associated with the withdrawal syndrome.

Methods
• Expt 1: Conditioned Place Aversion (CPA): Rats were exposed to two neutral, environmentally distinct chambers. During conditioning, animals were injected intraperitoneally (i.p.) with saline or morphine (10 mg/kg) followed by naloxone (2.5 mg/kg) 2 hrs later to precipitate withdrawal. Animals were then confined to one of the chambers. On the final test day, rats received no drugs and were allowed to explore both chambers. Time spent on each side was measured as percent of exploration.
• Expt 2: Withdrawal-potentiated Startle (WPS): Rats received a baseline startle test followed by systemic morphine injections (10 mg/kg) in the colony room. Animals were allowed to undergo spontaneous withdrawal, and then given a second startle test at 4 hrs. Acoustic startle was measured by presentation of 95 and 105-dB white noise bursts in a pseudorandom order. Two habituation sessions were given prior to morphine exposure. Morphine was administered each day for 7 days, and WPS was measured on days 1, 2, and 7.

Results

Experiment 1

Fig 1. Male HiS rats show more severe CPA than LoS males during morphine withdrawal.
• Experimental timeline. HiS and LoS females, as well as HiS males spent less time on the drug-paired side, indicative of a withdrawal effect. LoS males did not change in the amount of time spent on the drug-paired side.

Experiment 2

Fig 2. HiS males show greater morphine WPS than LoS males, but there are no phenotypic differences among females. A. Experimental timeline. B. HiS and LoS males do not differ significantly in WPS on day 1 of morphine administration, but HiS males increase in WPS on days 2 and 7 while LoS males show decreased WPS. Saline-treated controls do not exhibit significant changes in startle between baseline and posttests. C. Female HiS and LoS rats had similar WPS on all days, suggesting no differences in morphine withdrawal-induced anxiety.

Conclusions
• CPA: HiS males avoid and spend less time in the withdrawal-paired chamber than LoS males, suggesting that morphine withdrawal is more aversive for HiS males. Both HiS and LoS females avoid the withdrawal-paired chamber and spend a similar amount of time in the neutral chamber.
• WPS: HiS and LoS males have similar morphine WPS on the first day of morphine exposure. HiS males exhibit even greater startle amplitudes on day 2 and 7 of morphine, whereas LoS males show similar withdrawal levels on day 1, but this effect diminishes by day 7. HiS and LoS females exhibit similar levels of anxiety on all days of WPS.
• These findings indicate that HiS males experience more anxiety-like, aversive symptoms during morphine withdrawal than LoS males. Although LoS males start out showing similar anxiety as HiS males, after 7 days this effect disappears.
• This suggests that the relationship between saccharin consumption and severity of morphine withdrawal is present in males but not females, and that LoS males do not experience anxiety-like withdrawal symptoms after 7 days of morphine.
• Prior studies have shown that LoS female rats experience more severe ethanol withdrawal than HiS rats on measures of acoustic startle responses.7,8 Although the present experiments do not support such findings, this discrepancy could be due to different protocols of measuring acoustic startle and the drug of administration.
• The finding that HiS male rats, who self-administer more drugs of abuse than LoS males, have more pronounced withdrawal symptoms supports the idea that negative affect contributes to drug seeking and drug taking. Moreover, affective profiles of anxiety may play a role in prodromal to substance abuse, and further study of the HiS and LoS strains will help gain new insight on clinical applications for drug addiction.

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References