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Research report

The effects of light cycle phase on morphine-induced conditioned taste aversions in the Lewis, Fischer and Sprague-Dawley rat strains

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ABSTRACT

The present experiment investigated the effect of light cycle phase on morphine-induced conditioned taste aversions in the Lewis (LEW), Fischer (F344) and Sprague-Dawley (SD) rat strains. Separate groups of rats from each strain were trained during either the light phase or the dark phase on a procedure in which saccharin was paired with one of two doses of morphine (or vehicle). With 3.2 mg/kg morphine, strain differences were observed during the light phase, with F344 rats displaying a significantly stronger taste aversion than the LEW rats, who displayed a significantly stronger aversion than the SD rats. In contrast, during the dark phase, 3.2 mg/kg morphine produced comparable, moderately strong aversions in all strains. With 10.0 mg/kg morphine, F344 rats developed stronger aversions than either the LEW or SD rats in both phases of the light cycle. The effect of light cycle was most clearly seen in the SD rats, where stronger aversions were produced in the dark phase for both morphine doses. For the LEW rats, stronger aversions were produced in the dark as compared to the light only with the low dose of morphine. For the F344 rats, aversions of comparable strength were observed in both phases of the light cycle for both morphine doses. The finding that light cycle differentially affects morphine-induced taste aversions in these strains is consistent with what is known about strain differences in circadian patterns of corticosterone activity and with previous results relating corticosterone to morphine-induced taste aversions.

1. Introduction

The Fisher (F344) and Lewis (LEW) rat strains differ on a myriad of behavioral and physiological endpoints (for a review, see [20,29]). One area in which such differences have recently been reported is with taste aversion learning (for a review, see [29]). In the taste aversion preparation, an animal is given a novel solution to drink and injected with one of a number of compounds [9,28,33; see also 30]. Animals generally decrease consumption of the drug-associated solutions on subsequent exposure, indicating the aversive effects of the drug (though see [16] for an alternative interpretation). As noted, within this context, F344 and LEW rats differ. For example, aversions induced by morphine [23], nicotine [26] and alcohol [32] are relatively stronger in the F344 strain than the LEW strain. In contrast, aversions induced by cocaine are generally stronger in the LEW strain [14,16; though see 7,21,31]. Given that drug use is thought to be a function of the balance between a drug's rewarding and aversive effects, these reported differences between the LEW and F344 strains in drug-induced conditioned taste aversions have been interpreted as a potential genetic model of differential vulnerability to drug abuse (e.g. [7]).

It has been argued that the differences observed between F344 and LEW rat strains in their responses to drugs of abuse may be mediated in part by well-documented differences between these strains' hypothalamic-pituitary-adrenal (HPA) axis functioning [20; see also 15]. These differences in HPA activity may also be important for the reported differences in taste aversion learning between the two strains. Evidence for this is provided by the findings that (a) in an outbred rat strain (Sprague-Dawley), a saccharin solution previously paired with morphine elicits a corticosterone response and the magnitude of this response is positively correlated with the magnitude of the morphine-induced CTA [11], and (b) F344 and LEW rats show major between-strain differences in corticosterone activity, including the corticosterone stress response to aversive events [35,13,12]. Although the corticosterone response to morphine-paired saccharin has not been directly measured in F344 and LEW rats, it has been shown that these strains differ in their corticosterone response to morphine (administered i.p.), with LEW rats displaying a smaller response [2].

An important way in which F344 and LEW rats differ in corticosterone functioning is in how the activity of this hormone is influenced by circadian factors [8,36]. Although LEW and F344 rats

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display comparable corticosterone levels during the dark phase of the light:dark cycle, these levels fall and reach their lowest point during the earliest part of the light phase for LEW rats but remain elevated for F344 rats [36]. The circadian pattern of corticosterone activity observed in LEW rats (elevated in dark phase, reduced in light phase) is similar to that observed in outbred Sprague-Dawley (SD) rats [37]. These differential patterns of basal levels of corticosterone are functions of strain differences in the frequency and magnitudes of the brief pulses of corticosterone release observed during the different phases of the light cycle [36]. Specifically, LEW rats display corticosterone pulses that are more frequent and of higher magnitude during the dark phase as compared to the light phase. In contrast, for F344 rats there is no difference in the frequency or magnitude of these corticosterone pulses over the phases of the light cycle. Again, SD rats show a pattern of pulsatile release of corticosterone that is similar to that of LEW rats [37].

In addition to the differential influence of light cycle on basal corticosterone levels in LEW and F344 strains, circadian factors may also differentially affect the stress-induced corticosterone response in these strains. Windle et al. [36,37] found that LEW rats only display increased corticosterone release in response to stress during the rising phase of a corticosterone pulse (i.e. when basal corticosterone levels are increasing), while the stress-induced increase in corticosterone in F344 rats is independent of pulse phase. Because corticosterone pulses are more frequent and of higher magnitude during the dark phase as compared to the light phase in LEW rats, increased corticosterone release in response to stress should be more likely to occur during the dark phase in this strain. In contrast, F344 rats would be expected to show a similar corticosterone stress response during both the light and dark phases since the frequency and magnitude of corticosterone pulses do not vary over the course of the day in this strain.

Given the findings that morphine-induced CTA is associated with the corticosterone response in outbred rats [11] and the strain differences in circadian patterns of corticosterone activity described above [8,36,37], it might be expected that differences in aversion learning between the two strains would be a function, in part, of the specific cycle in which aversion learning is assessed. Accordingly, the present experiment examined the effect of circadian cyclicity on morphine-induced taste aversions in the F344 and LEW rat strains. For comparison, aversions in these two strains were compared to the outbred Sprague-Dawley rat trained and tested under similar conditions.

2. Materials and methods

2.1. Animals

Fifty LEW, 50 F344, and 50 SD female rats, approximately 6 weeks old and weighing on average 148.9 g (S.E.M. = 2.3 g), 131.6 g (S.E.M. = 0.6 g), and 192.1 g (S.E.M. = 2.4 g), respectively, were obtained from Harlan Sprague-Dawley (Indianapolis, IN). All animals were housed in groups of four and were left undisturbed in their plastic bins (48 cm × 27 cm × 20 cm) until they reached 9 weeks of age. The room in which they were located was maintained at 23 °C and on a 12:12-h light:dark cycle (see below). Water and food were available *ad libitum*. Guidelines established by the Institutional Animal Care and Use Committee at American University were followed at all times.

2.2. Drugs

Morphine sulfate (generously supplied by NIDA) was prepared in a 3 mg/ml solution in 0.9% sodium chloride (Biofluids, Biosource International). Morphine and vehicle control injections were administered subcutaneously. Sodium saccharin (0.1%; Sigma, St. Louis, MO) solution was prepared as a 1 g/l solution in tap water.

2.3. Procedure

2.3.1. Light cycle acclimatization

At 9 weeks old, rats of each strain were randomly assigned to either the light or the dark condition. Rats in the two conditions were housed in separate rooms off the same hallway. Each room had a different 12:12-h light:dark cycle. For rats in the "light" condition, the 12-h light phase began at 08:00 h. For rats in the "dark" condition, the 12-h light phase began at 20:00 h. Rats in both conditions were individually housed in stainless steel, wire-mesh cages on which a graduated Nalgene bottle could be placed. During this time, access to food and water was unlimited. Throughout the experiment, during those brief periods (typically less than 10 min at a time) when the experimenter entered the dark room, a red light bulb that provided very low level illumination was turned on so that the experimenter could see. Rats spent 3 weeks acclimating to these new conditions.

2.3.2. Water deprivation

Following 24 h of water deprivation, all subjects were given 20-min access to water each day for 21 consecutive days. For rats in both light cycle conditions, water access was given at 12:00 h. This access was 4 h into the light phase for those rats in the light condition and 4 h into the dark phase for those rats in the dark condition.

2.3.3. Conditioning

On day 1 of this phase, all subjects were given 20-min access to a novel saccharin solution at the scheduled time, i.e. 12:00 h. Immediately after saccharin access, subjects within each strain and light cycle condition combination were ranked according to their saccharin consumption and assigned to one of the two morphine doses, 3.2 or 10.0 mg/kg, or vehicle. Assignments were made such that mean saccharin consumption was comparable over doses. Subjects were then given a subcutaneous injection of the appropriate morphine dose or saline equivolume to the highest dose of morphine. On the following three water-recovery days, all subjects were given 20-min access to water. This sequence of alternating a single conditioning day with 3 water-recovery days was repeated five times.

2.3.4. Conditioned Taste Aversion Test

On the day following the final water-recovery session, all subjects were given 20-min access to saccharin in a test of the aversion to saccharin. This test was the same as a conditioning trial except that no injections were given following saccharin access.

2.4. Data analysis

For determinations of statistical significance, $\alpha = 0.05$. To assess strain differences in body weights, a one-way ANOVA followed by Tukey post hoc tests was performed. Baseline water consumption prior to conditioning was analyzed by performing a 3 × 2 (Strain × Light Cycle) ANOVA on the water consumption data from the day prior to the first conditioning trial. The resulting significant Strain × Cycle interaction was further investigated by performing (1) separate one-way ANOVAs followed by Tukey post hocs for each phase of the light cycle separately, and (2) independent *t*-tests comparing light with dark for each strain separately. The type 1 error rate for this collection of *t*-test was held at ≤0.05 using the Benjamini–Hochberg procedure [3].

To analyze baseline (i.e. before conditioning) saccharin consumption, a 3 × 3 × 2 (Strain × Dose × Light Cycle) factorial ANOVA was performed on the saccharin consumption data from Trial 1 (i.e. before saccharin was paired with an injection). Tukey multiple comparisons were used to further investigate significant main effects.

Pearson *r* correlation coefficients were computed for the correlation between Trial 1 saccharin consumption and body weight on that day within each Strain × Light Cycle combination.

Because the strains differed in baseline saccharin consumption, the conditioning data were analyzed in two different ways. First, to characterize the acquisition of CTA, separate 2 × 3 × 6 (Light Cycle × Dose × Trial) factorial ANOVAs were performed on the absolute consumption data for each strain. Significant ANOVA interactions were followed by 2 × 6 (Cycle × Trial) factorial ANOVAs performed at each dose separately.

The second way of analyzing the data allowed for between-strain comparisons by normalizing saccharin consumption during Conditioned Taste Aversion Test. This was accomplished by converting saccharin consumption during the test trial to a percentage of Trial 1 (i.e. baseline) consumption (i.e. [saccharin consumption during CTA test/saccharin consumption during Trial 1] × 100). A 3 × 2 × 3 (Strain × Cycle × Dose) factorial ANOVA was performed on this percentage measure. There were significant ANOVA interactions (described below) that were further investigated by performing separate 3 × 2 (Strain × Cycle) factorial ANOVAs for each dose. Significant main effects of Strain or Cycle in the absence of significant interactions were followed by Tukey multiple comparisons, where appropriate. Significant Strain × Cycle interactions were followed by (1) one-way ANOVAs performed separately for each light cycle condition (which were followed by Tukey multiple comparisons if *F* was significant) and (2) between-groups *t*-tests comparing, within each strain, subgroups trained in the light phase with those trained in the dark phase (i.e. LEW-light vs. LEW-dark, F344-light vs. F344-dark, and SD-light vs. SD-dark). The type 1 error rate for this family of three *t*-tests was held at ≤0.05 using the Benjamini–Hochberg procedure [3].