



Adolescent Stress Enhances Morphine Conditioned Place Preference in Rats

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Introduction

Overstimulation of stress-response systems associated with childhood adversity has been shown to alter neural functioning and cause physical changes in the brain (Chocyk, 2013). Research widely supports the finding that those who experience childhood adversity are at higher risk for negative physical and mental health outcomes, including substance abuse disorders (Marquez et al., 2013). Although stress during adulthood may have similar consequences, early-life stress may have a stronger impact because of its co-occurrence with important advances in neurodevelopment (Tyrka, Burgers, Philip, Price, & Carpenter, 2013). Whereas previous research has demonstrated enhanced drug reward following adult stress (e.g., Der-Avakian et al., 2007), the present study investigated the impact of peripubertal stress on morphine reward in a place conditioning procedure.

Hypothesis

Subjects in the peripubertal Stress (S) condition were predicted to show enhanced morphine conditioned place preference in comparison to animals in the No Stress (NS) condition.

Method

- **Subjects** – 12 adolescent male Sprague-Dawley rats were obtained at postnatal day (P) 25.
- **Phase 1 (Stress)** (Adapted from Marquez et al., 2013)
 - **Elevated Platform (EP)** – Subjects were placed on a 12 x 12 cm Plexiglas platform affixed to a 95 cm tall support column illuminated from the side by a bright light for 25 minutes.
 - **Predator Odor (PO)** – Subjects were placed in individual polycarbonate cages containing a 10 x 10 cm cloth saturated with 10 mls of red fox urine (Wildlife Research Center) for 25 minutes.
 - **Procedure** – Half the animals were assigned to the NS condition and the other half to the S condition. Beginning on P28, the S group was exposed to EP and PO stressors following an unpredictable schedule (see Table 1).

Method (cont.)

Day	Stressor
1	EP only
2	EP, PO
3	PO, EP
4	PO only
5	None
6	EP only
7	EP, PO
8	EP only

Table 1. Schedule of unpredictable stressor exposure for the S group (Marquez et al., 2013).

- **Phase 2 (Place conditioning)**
 - **Apparatus** – The place conditioning chamber consisted of two sides, a Black/Grid side (black walls with a grid floor) and a White/Hole side (white walls with a hole floor), separated by a partition.
 - **Drug** – Subjects received a moderate intraperitoneal dose of 15 mg/kg morphine (15 mg/ml concentration). Saline was used at an equivalent volume, 1 ml/kg.
 - **Procedure**
 - **Pre-test** – At P38 subjects were placed in the apparatus and allowed free movement through the partition for 15 minutes. In accordance with a biased procedure, animals were assigned to receive morphine on the initially non-preferred side of the apparatus (drug-paired side, CS+) and saline on the initially preferred side (non-drug paired side, CS-).
 - **Training** – Subjects received eight days of training following pre-test. Alternating by day, animals received either morphine on the CS+ or saline on the CS- (15 minute trials). Animals were restricted to the appropriate side of the apparatus by the partition.
 - **Post-test** – A post-test identical to the pre-test followed training.

Results

- **Time on Non-Preferred Side**
 - A 2 (stress) x 2 (test) mixed ANOVA revealed a significant main effect for stress, $F(1, 10) = 6.854$, $p = .026$.
 - A main effect of test (pre vs. post) was also found, $F(1, 10) = 49.47$, $p < .001$.

Results (cont.)

- A stress by test interaction was found, $F(1, 10) = 6.981$, $p = .025$, indicating a unique effect of stress from pre- to post-test. Subsequent post hoc Tukey tests revealed that S and NS groups did not differ significantly at pre-test; however, the groups did differ at post-test, with S subjects showing stronger conditioned place preference (CPP) than NS subjects. In addition, CPP was observed in both groups as evidenced by a significant increase in time on the non-preferred side between pre- and post-test in both S and NS subjects (see Figure 1).
- Overall, findings indicate significantly stronger morphine CPP in the S group than in the NS group.

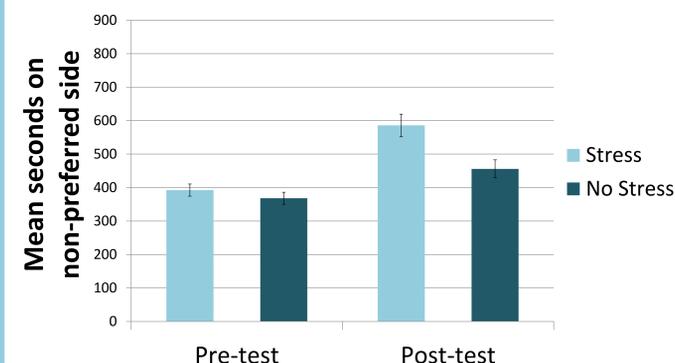


Figure 1. Mean seconds on the non-preferred side of the place conditioning apparatus from pre- to post-test in both S and NS subjects. Animals in the S show stronger morphine CPP at post-test.

- **Activity**
 - A 2 (stress) x 2 (drug) x 4 (trial) mixed ANOVA revealed no main effect of stress on activity $F(1, 10) = 2.042$, $p = .183$.
 - There was, however, a main effect for drug, $F(1, 30) = 24.149$, $p = .001$.
 - An interaction between drug and activity day was found, $F(3, 30) = 7.786$, $p = .001$. Post hoc Tukey tests indicated significantly lower activity on morphine training day one when compared to saline training day one. However, comparisons between drug conditions were not significant for any other training day. Within morphine days, day one activity was significantly lower than day four; no other comparisons within morphine or saline days were significant (see Figure 2).

Results (cont.)

- Overall, activity results indicate habituation over training days to the motor-suppressing effects of morphine.

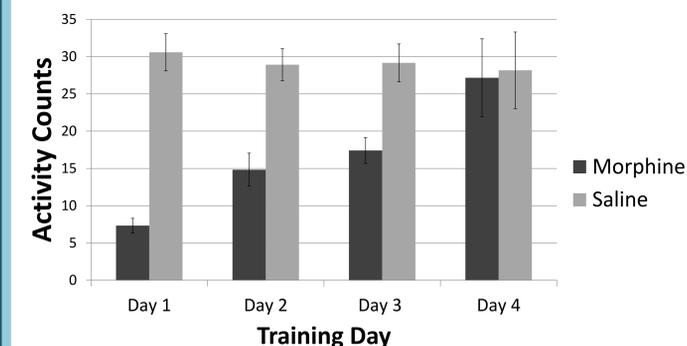


Figure 2. Mean activity counts for morphine and saline training days. Outcomes indicate habituation to morphine's motor-suppressing effects.

Discussion

- The present study indicates that peripubertal stress increases morphine CPP. This finding expands on past research demonstrating that adult stress potentiates morphine CPP (e.g., Der-Avakian et al., 2007).
- Results also support previous clinical research showing that childhood adversity can increase risk of substance abuse (e.g., Myers, McLaughlin, Wang, Blanco, & Stein, 2014).
- The potential for peripubertal stress to enhance morphine CPP suggests that reduction of childhood adversity (such as abuse and neglect) is an important element of substance abuse prevention efforts.

Future Directions

- As the present study utilized a limited sample size, future research should explore the relationship between stress, age at time of stress, and morphine CPP using larger sample sizes.
- Future research should also investigate the influence of age at time of stress on drug reward by comparing groups stressed at different stages of development.
- The neurobiological impact of peripubertal stress on morphine CPP should also be examined.