



Effects of Early-Adolescent, Mid-Adolescent, or Adult Stress on Morphine Conditioned Place Preference

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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). Though previous research has demonstrated enhanced drug reward following adult stress (e.g., Der-Avakian et al., 2007), the present study directly compares adult and adolescent stress to investigate any selective effect on morphine reward in a place conditioning procedure.

Method

Subjects – 48 adolescent male Sprague-Dawley rats were obtained at postnatal day (P) 20.

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 under bright illumination for 25 min.

- **Predator Odor (PO)** – Subjects were placed in individual polycarbonate cages containing a filter paper saturated with 10 μ l of synthetic fox odor trimethylthiazoline for 25 min.

- **Procedure** – Rats were randomly assigned to the early-adolescent, mid-adolescent, or adult group. Each age group was then further divided into Stress (S) or No Stress (NS) conditions. Following an unpredictable schedule (see Table 1), S rats were exposed to the stress procedure beginning on P21, P35, or P60 depending on age group (see Figure 1).

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).

Method (cont.)

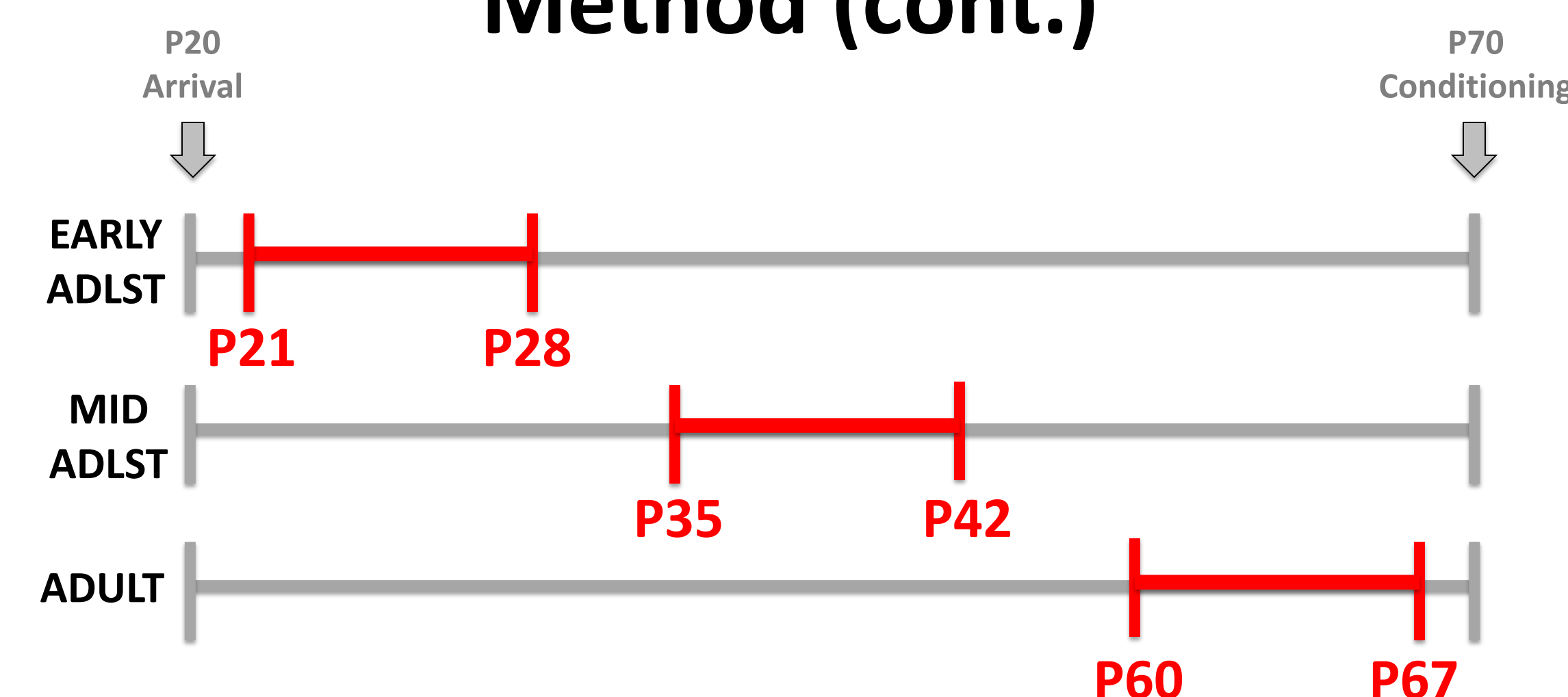


Figure 1. Schedule of stress exposure for S rats in each age group.

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 P70 subjects were placed in the apparatus and allowed free movement through the partition for 15 minutes. Following a biased procedure, rats received 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** – Eight days of training followed the 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** – Identical to the pre-test.

Results

Time on CS+

- A 2 (stress) x 2 (test) x 3 (age group) mixed ANOVA revealed a test by age interaction [$F(2, 42) = 4.59, p < .05$]. Post hoc Tukey tests confirmed a significant increase in time on the CS+ from pre- to post-test but found no differences between age groups.

- A main effect of test (pre vs. post) was also found [$F(1, 42) = 115.90, p < .001$].

Results (cont.)

- Findings indicate overall conditioned place preference (CPP) but no effect of stress or age of stress exposure.

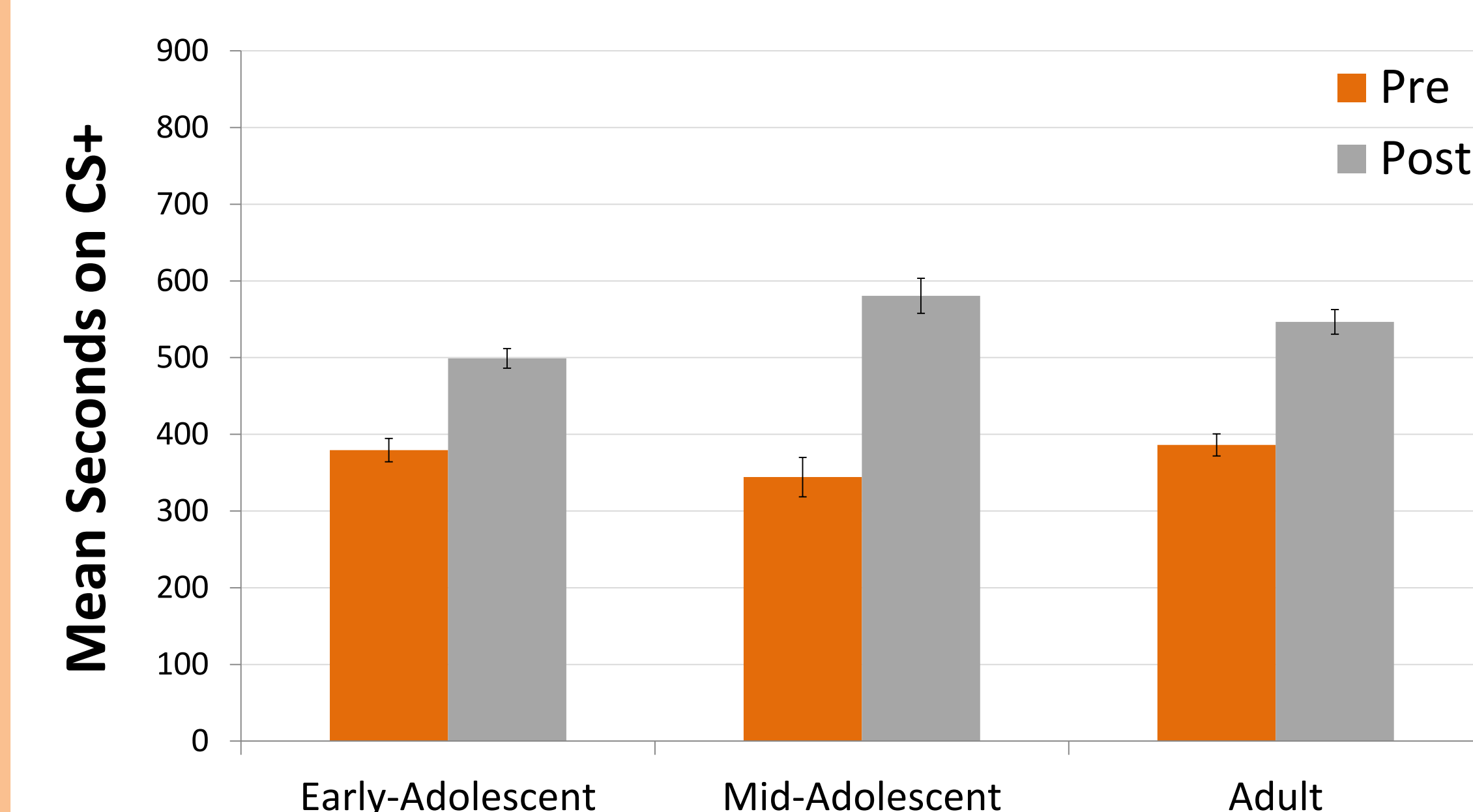


Figure 2. Mean seconds on the CS+ from pre- to post-test. Results indicate significant overall conditioned place preference but no effect of stress or age group.

Activity

- A 2 (stress) x 2 (drug) x 3 (age group) x 4 (trial) mixed ANOVA revealed a main effect of trial [$F(3, 126) = 5.06, p < .005$].

- An interaction between trial and age [$F(6, 126) = 3.29, p < .05$] was found. Post hoc Tukey tests indicated a significant difference between the early-adolescent and adult groups on trial 1.

- Though an interaction was found between stress and age [$F(2, 42) = 5.68, p < .05$], post hocs were not significant.

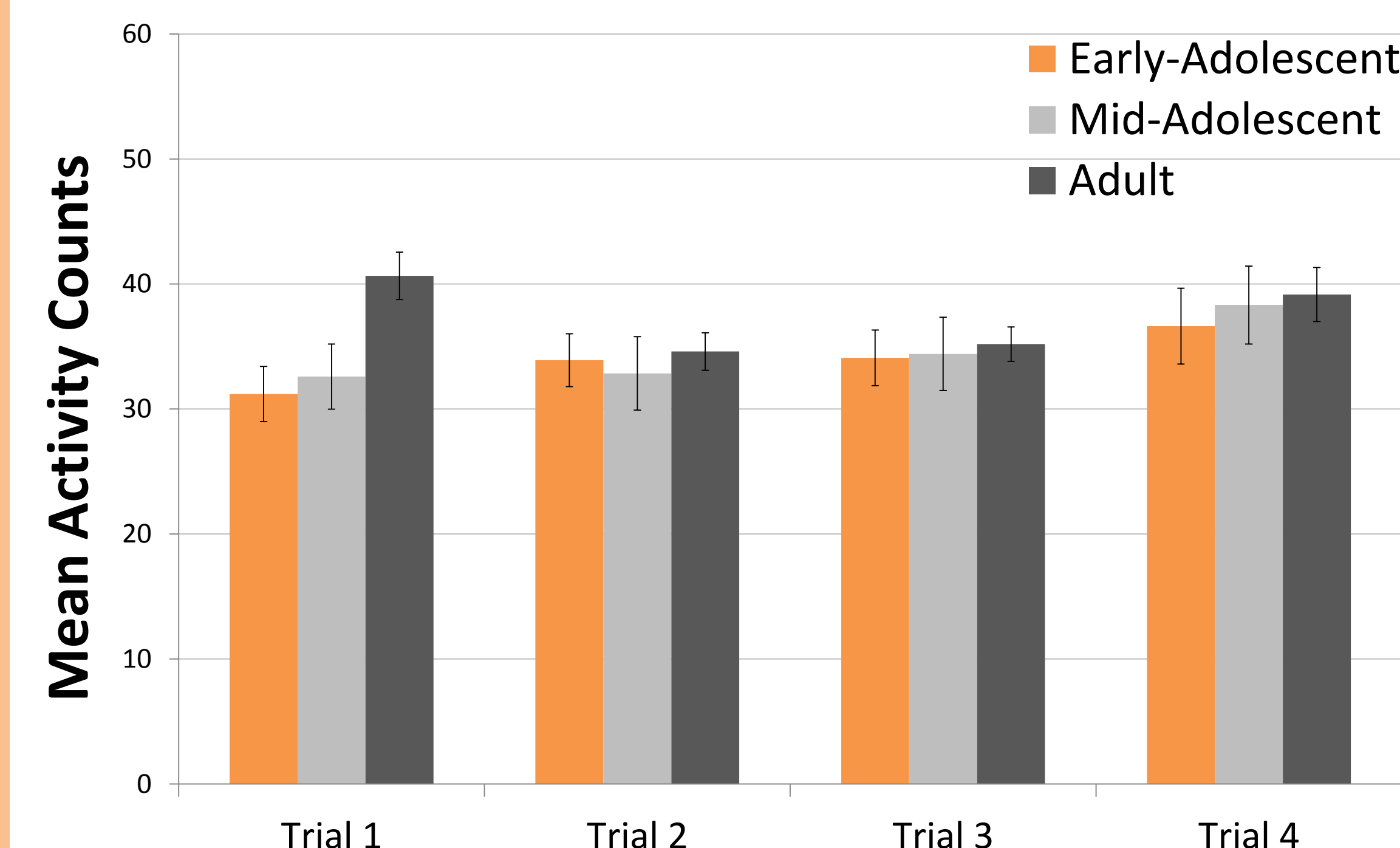


Figure 3. Activity by trial for each age group. A significant difference between the early-adolescent and adult groups was found on trial 1.

- An interaction between drug and trial was found [$F(3, 126) = 101.24, p < .001$]. Tukey tests revealed:

- Sal vs. morphine: Sal > morphine on Trial 1; sal < morphine on Trials 3 & 4.

Results (cont.)

- Within morphine trials: Trial 1 < Trials 2, 3, & 4; Trials 2 & 3 < Trial 4.

- Within saline trials: Trial 1 > Trials 2, 3, & 4.

- Overall, activity results indicate habituation over trials to the motor-suppressing effects of morphine, and a reduction in activity over saline trials.

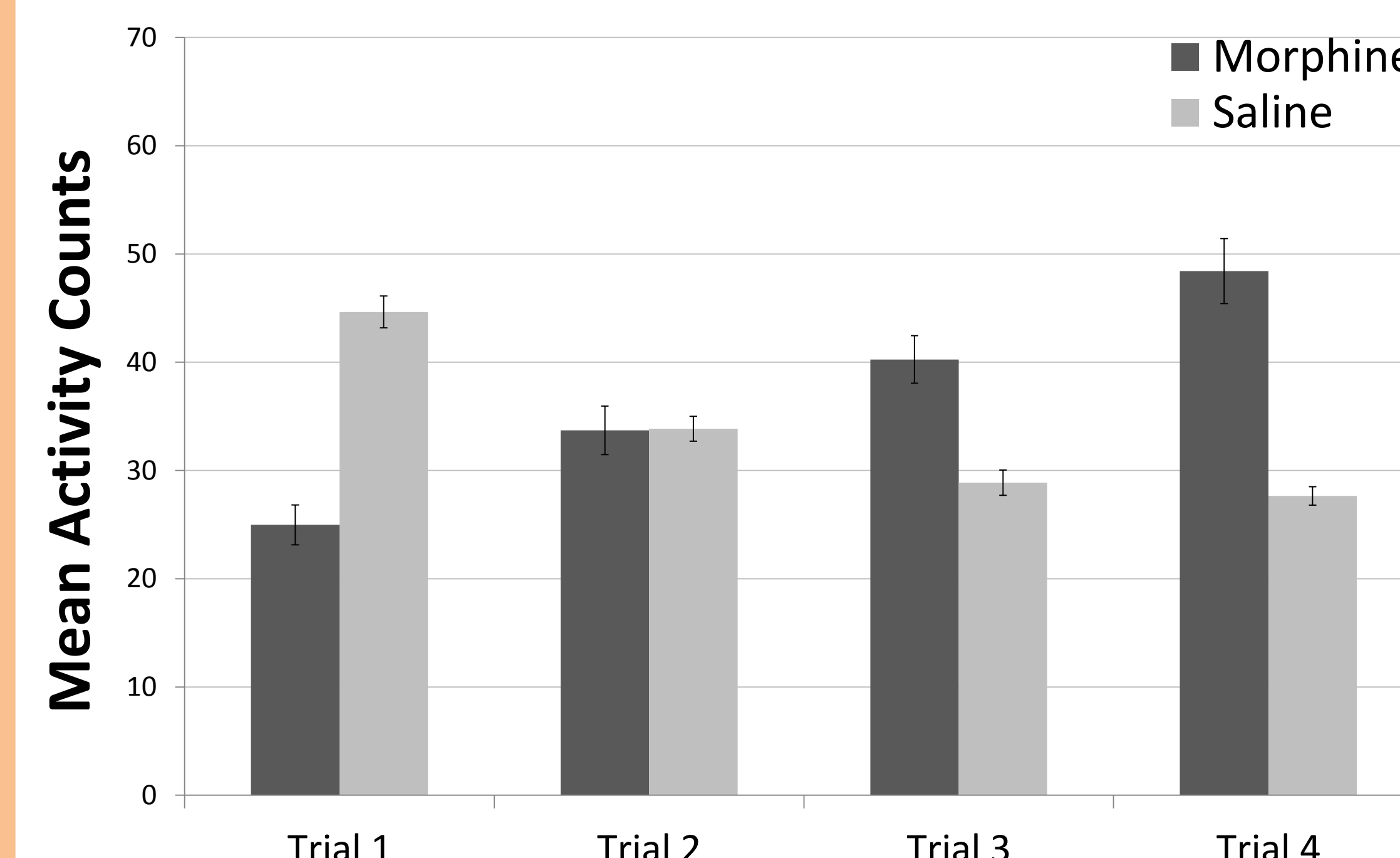


Figure 4. Mean activity counts for morphine and saline training days. Outcomes indicate habituation to morphine's motor-suppressing effects and a reduction in activity over saline trials.

Conclusions

- Robust CPP was found regardless of age or stress condition, indicating no selective effect of stress on opiate reward as measured by the place conditioning procedure.

- For activity, adults were higher than early-adolescents in the first trial. Additionally, habituation to morphine's motor-suppressing effects was found over trials, while saline activity decreased over trials.

- The lack of significant findings may indicate the stress paradigm used has no effect on morphine CPP.

- However, pilot work in our laboratory found a stress effect in adolescents (stronger CPP in S group) when place conditioning commenced 48 hours after stress exposure. The stress procedure used may therefore have a short-term effect on morphine CPP that selectively impacts adolescents.

- Future work could adapt this study to investigate the short-term effect of stress by starting conditioning just after the conclusion of the stress phase.

- Future work could confirm the effectiveness of the stressors by evaluating biological markers of stress such as corticosterone release and puberty delay.