



Unusual Locomotor Response to Morphine in BTBR T^{tf}/J Mice



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Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder associated with several cardinal traits including social interaction deficits, communication deficits, and high anxiety levels. It has also been argued that motor coordination deficits should also be considered a core feature (Fournier et al., 2010). The BTBR T^{tf}/J (BTBR) strain of mice serve as a model for autism, exhibiting many of these core features (McFarlane et al., 2008) and differing physiologically in many ways from controls. Previous studies in our laboratory have shown that BTBR mice have diminished levels of striatal dopamine (DA). Opiate induced motor hyperactivity is thought to result from increased striatal dopamine output (Kuschinsky & Hornykiewicz, 1974). Additionally, the mu receptor has also been implicated in regulating social reward as have increased dopamine levels in the striatum (Becker et al.; de la Fuente-Fernández et al., 2002). Becker et al. have postulated that ASD might stem from a fundamental dysfunctioning of the reward and motivation pathway. In the present study, we investigated the differential effect of morphine on several different measures of general locomotor activity in BTBR mice and in C57BL/6 (B6) control mice in a dose-dependent analysis. We hypothesized that diminished striatal DA in the BTBR mice will translate to diminished locomotor response to systemic opiate treatment, relative to B6 controls.

Methods

Animals: Adult male BTBR T^{tf}/J and C57BL/6J mice (40-90 days old) were bred in the laboratory of Dr. Hewlet McFarlane at Kenyon College (Gambier, OH) from stock mice purchased from The Jackson Laboratory (Bar Harbour, Michigan, USA). Mice were group-housed, kept on a reverse 12h: light cycle, and tested in a red light environment. Food and water were available *ad libitum* except during testing procedures.

Drug Treatment:

Mice received an injection of morphine (10mg/kg body weight, 20 mg/kg, or 30 mg/kg) or saline. All treatments were administered intraperitoneally.

Open Field Test:

Mice were brought into the testing room and individually placed in the center of an open field chamber for 10 minutes for habituation. Mice were then taken out of the chamber one at a time to receive drug treatment. Recording began immediately after injection and lasted 60 minutes. The open field data were analyzed using a one-way repeated measure analysis of variance and two-way analysis of variance.

Note: Testing took place between 9:00am and 4:00pm each day in order to reduce the possibility of circadian rhythm effects.

Results

Total Distance Traveled

Figure 1. shows a main effect of dose for B6 mice where all doses are significantly different from each other (RM one-way ANOVA $F=61.70$, $p<0.0001$, $df=47$). 20 mg/kg elicited the greatest increase in total distance from baseline levels for B6 mice (RM one-way ANOVA, $F=110.0$, $p<0.0001$, $df=47$). There is also a main effect of dose for BTBR in total distance traveled. All doses resulted in significantly different distance traveled except between saline and 30 mg/kg and between saline and 20 mg/kg (RM one-way ANOVA $F=24.95$, $p<0.0001$, $df=47$). Further, there was a significant difference between the total distance traveled for B6 and BTBR mice for both the 20 mg/kg and 30 mg/kg dose treatment groups (Two-way ANOVA, $F=24.95$, $p<0.0001$, $df=56$).

Vertical Activity

Figure 2. shows a main effect of strain (Two-way ANOVA, $F=13.67$, $p<0.01$, $df=56$) and a main effect of dose groups (Two-way ANOVA, $F=3.76$, $p<0.05$, $df=56$) on vertical activity.

Center Distance

Figure 3. shows a main effect of treatment (Two-way ANOVA, $F=13.47$, $p<0.01$, $df=56$) but not a main effect of strain (Two-way ANOVA, $F=1.08$, $p>0.05$, $df=56$) on the distance traveled in the center of the open field apparatus.

Figures and Results

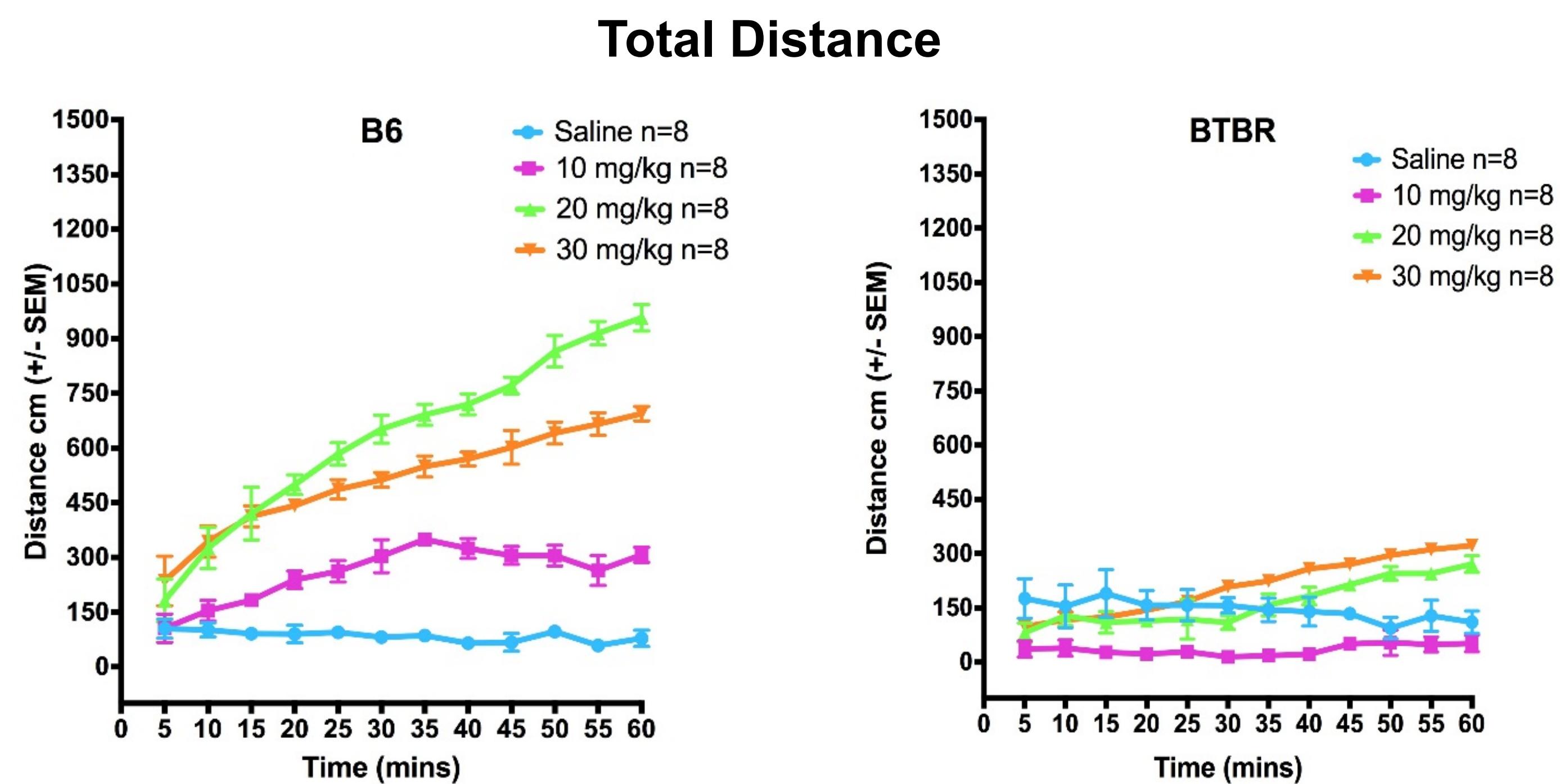


Figure 1.

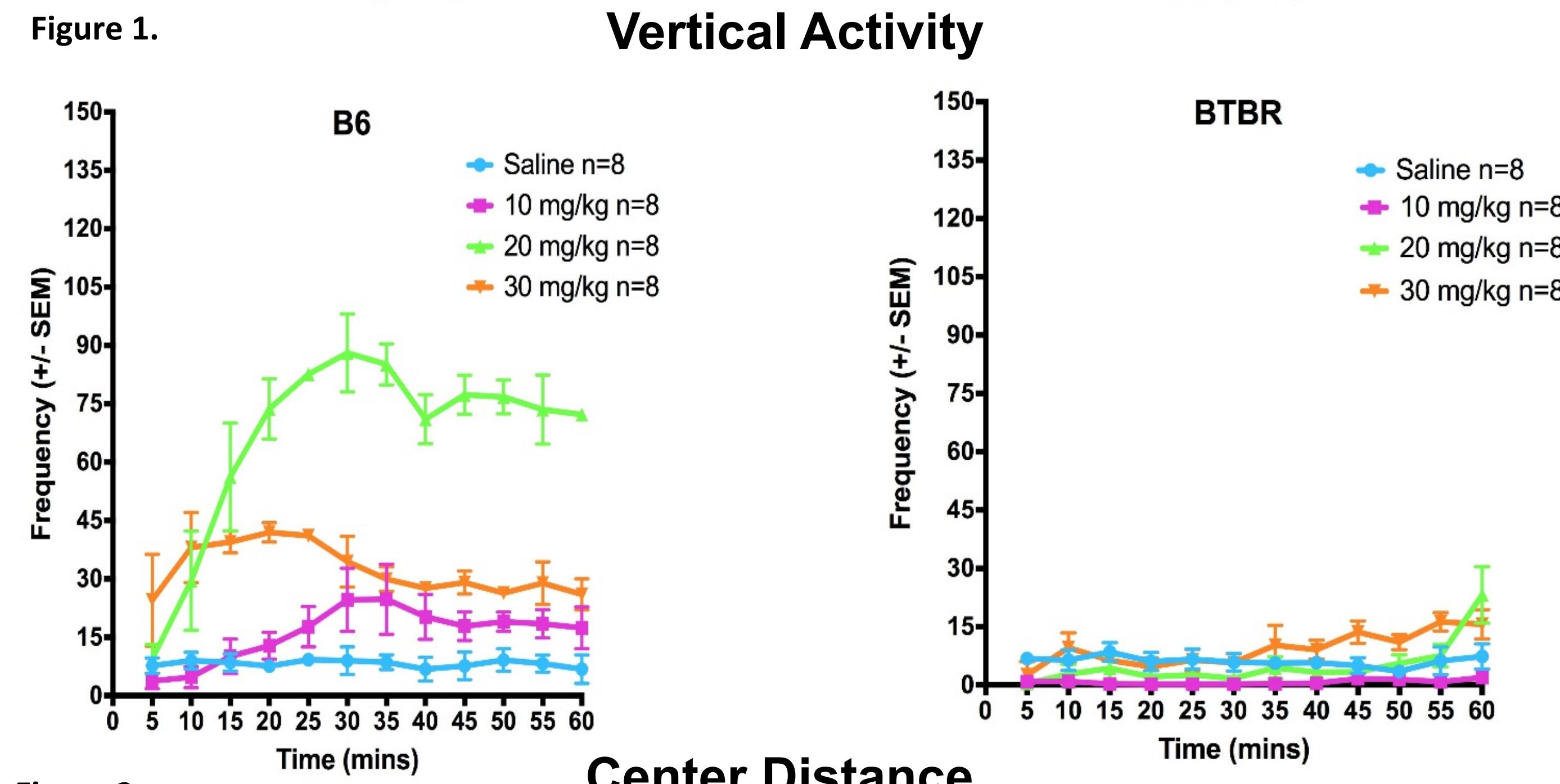


Figure 2.

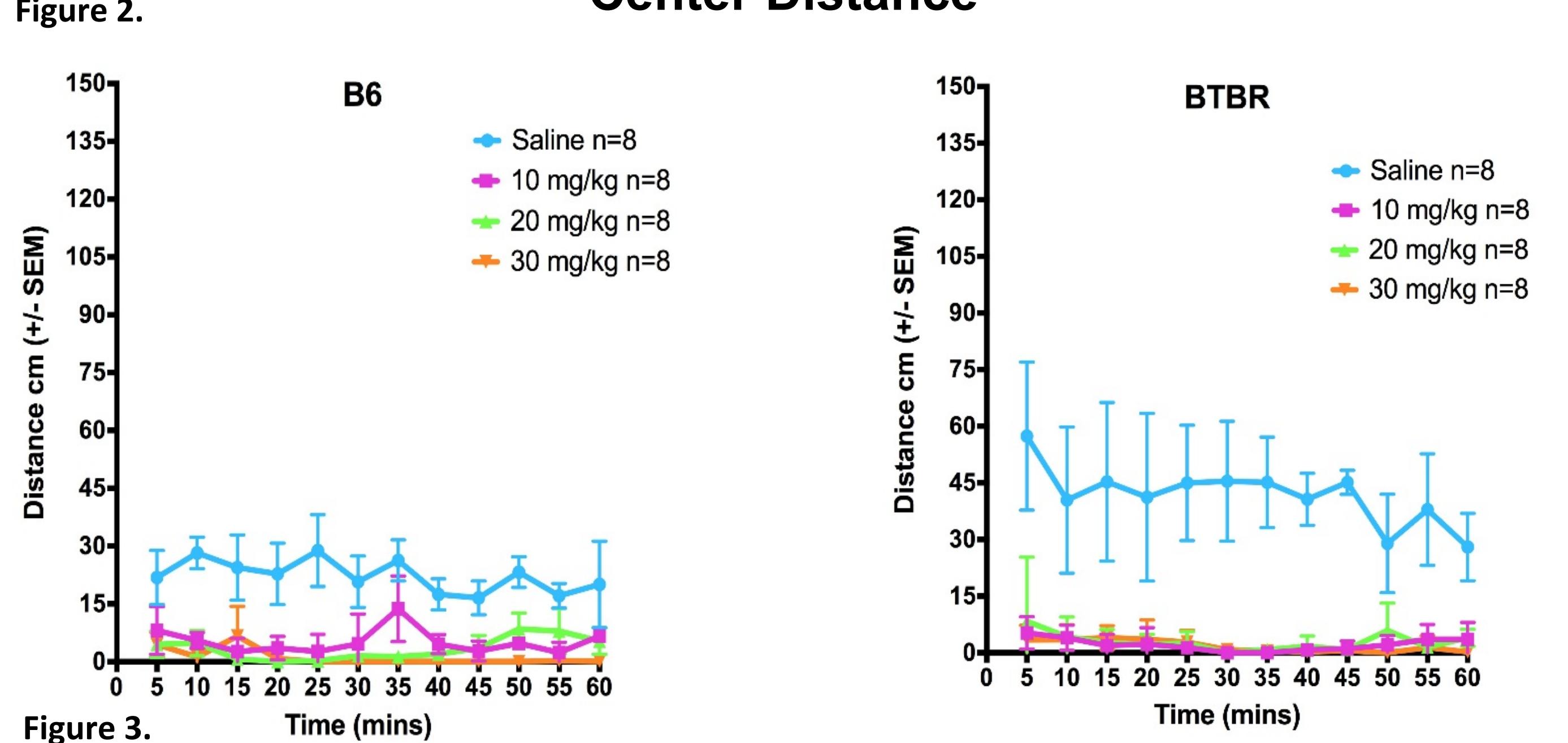


Figure 3.

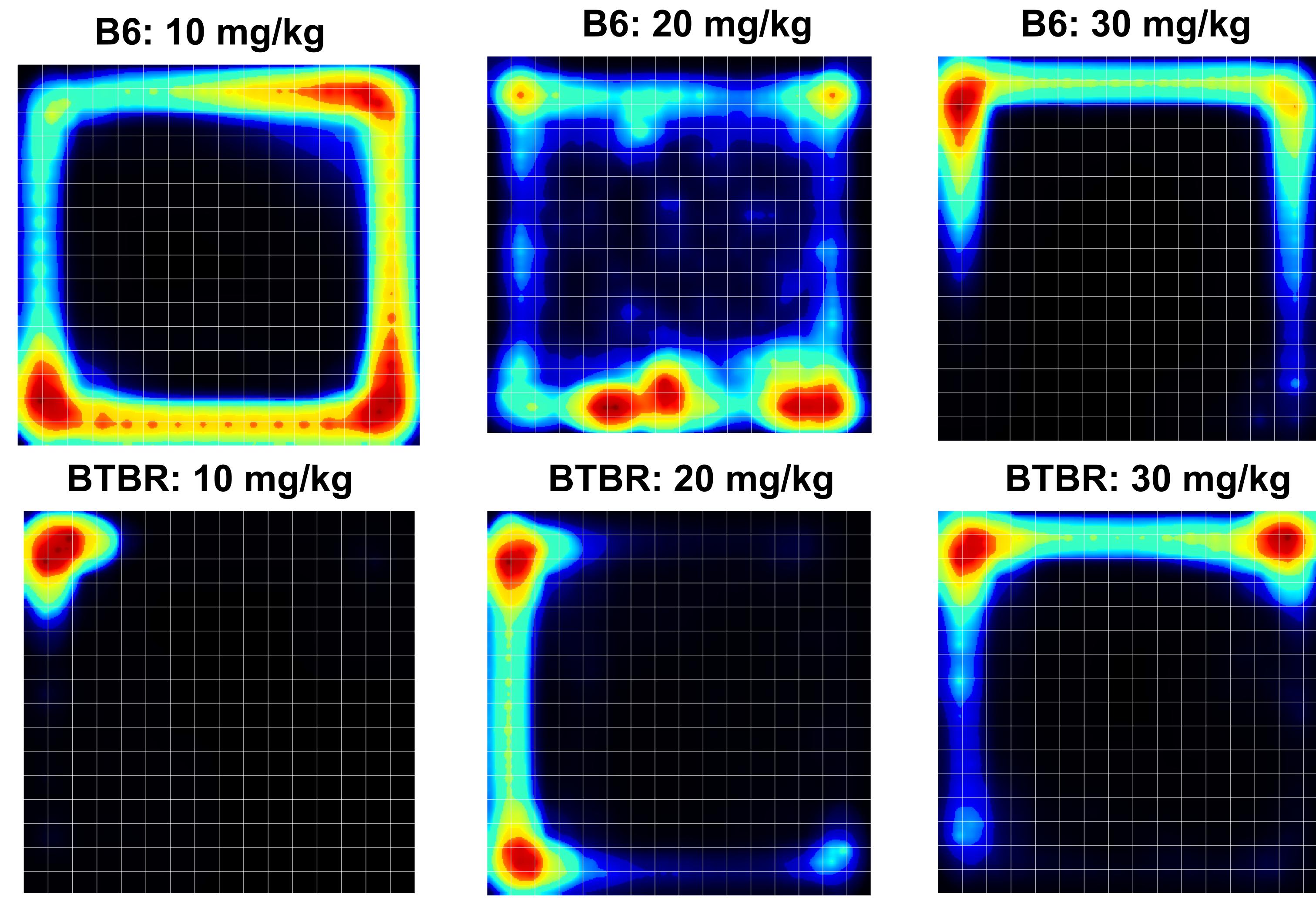


Figure 4. Heat maps show the amount of time a mouse spends in an area of the apparatus. Red represents the greatest amount of time, followed by yellow, and with blue representing the least time.

Figures continued

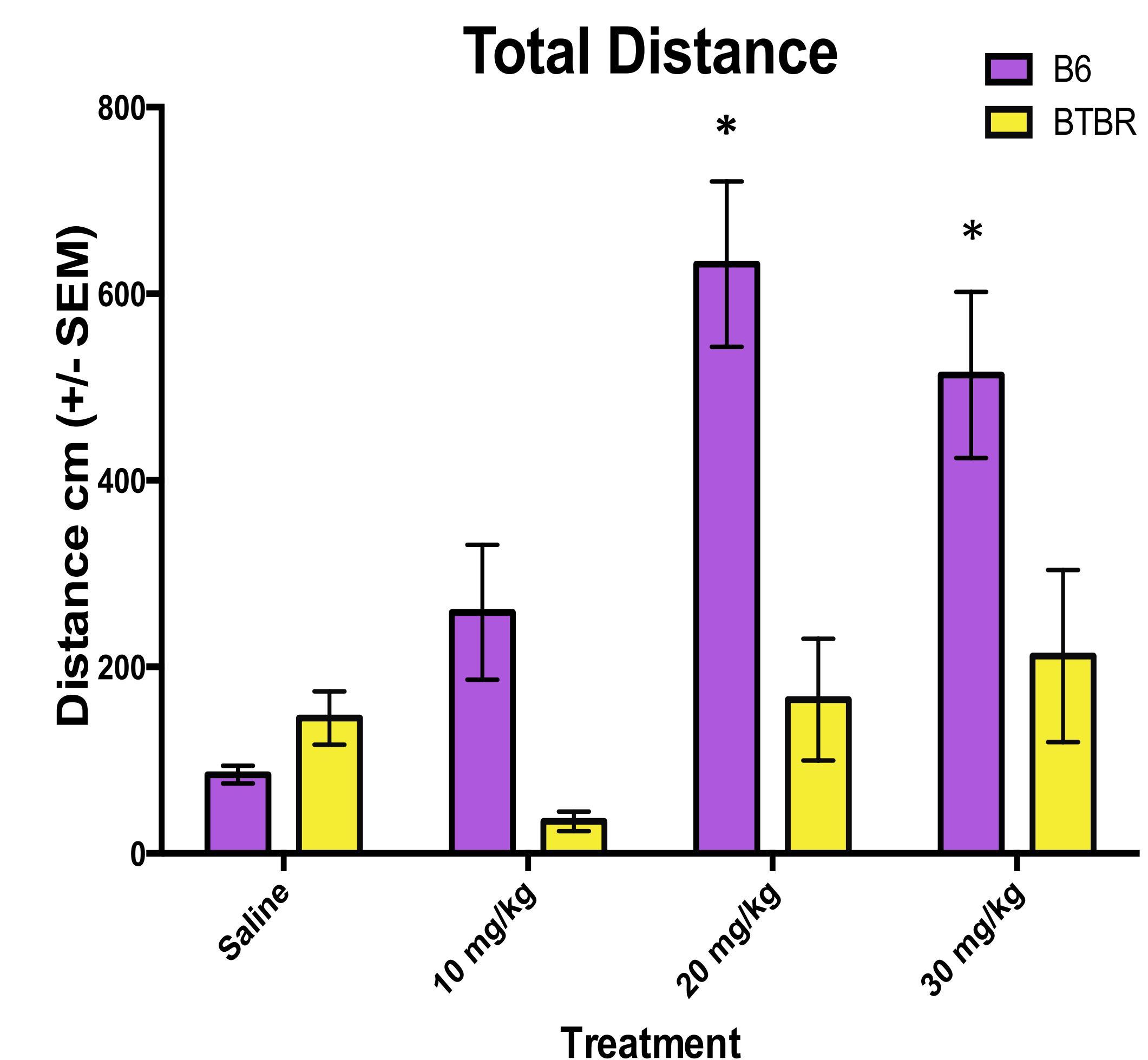


Figure 5. *Indicates significant difference between strains

Discussion

The results of this study demonstrate that the BTBR mice display both a delayed and muted response of hyperactivity to higher doses of morphine (20 and 30 mg/kg). It is possible that the cause of these differences are created by something inherently dysfunctional with the mu receptor system in BTBR's, such as a lower binding affinity to these receptors or fewer receptors, compared to controls. However, because we know BTBR's have less dopamine in the striatum, these responses might be due to alterations in the dopaminergic system in BTBR mice.

Future studies will focus on determining the involvement of the D2 dopamine receptor in the unusual locomotor responses seen in the BTBR mice. D2 receptors have high expression patterns in the striatum where they are strongly implicated in motor control. We expect to find decreased levels of this receptor in the striata of BTBR compared to B6 mice as D2 antagonists, analogous to having fewer receptors, tend to induce cataplexy similar to BTBR locomotion in this study. This will give us insight into the ways BTBR mice differ physiologically from normal mice.

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