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Effects of Neonatal Tactile Stimulation on Thermal Nociceptive Responses in WAG/Rij Rats

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Abstract

Background

Absence epilepsy is frequently accompanied by neuropsychiatric comorbidities and altered pain-related processing, suggesting shared neurobiological mechanisms. The WAG/Rij rat is a well-established genetic model that exhibits enhanced responsiveness to nociceptive stimuli. Early-life sensory experiences, particularly tactile stimulation, can modulate neural development and may influence nociceptive processing. This study investigated the long-term effects of early-life tactile stimulation on thermal nociceptive responses in male and female WAG/Rij rats.

Methods

Three groups were studied: Wistar controls, untreated WAG/Rij rats, and WAG/Rij rats receiving tactile stimulation during postnatal days 8–14. In adulthood, thermal nociceptive responses were assessed using the hot plate test at 54 ± 0.5°C and 52.5 ± 0.5°C. Locomotor activity was evaluated to control for motor confounds.

Results

At 54 ± 0.5°C, no group differences were observed in either sex. At 52.5 ± 0.5°C, WAG/Rij rats showed shorter response latencies than Wistar controls in females ($p = 0.0057$) and males ($p = 0.0329$). Tactile stimulation did not alter responses in females ($p = 0.2898$), but increased response latencies in males compared to untreated WAG/Rij rats ($p = 0.0001$), with no difference from Wistar controls ($p = 0.0605$). Locomotor activity did not differ between groups.

Conclusion

Early-life tactile stimulation produces long-term, sex-dependent effects on thermal nociceptive responses in WAG/Rij rats, particularly under lower-intensity stimulation. These findings highlight the role of early sensory experience in shaping nociceptive processing in genetically susceptible conditions.

Introduction

Absence epilepsy (AE) is a form of genetic generalized epilepsy characterized by brief, non-motor seizures associated with transient impairment of consciousness and responsiveness [1]. AE is a disorder accompanied by cognitive, behavioral, and affective comorbidities, including attentional deficits, executive dysfunction, anxiety, and depression [2, 3]. In parallel, epidemiological and clinical studies indicate that individuals with epilepsy report a higher prevalence of pain-related symptoms compared to the general population, suggesting that altered pain processing may represent an important yet

underexplored comorbidity of epileptic disorders [4, 5]. These observations point to potential shared neurobiological mechanisms underlying epilepsy and pain perception.

The WAG/Rij rat strain is a well-established genetic model of absence epilepsy that shows spontaneous spike–wave discharges on EEG recordings and exhibits comorbid depressive-like phenotypes [6, 7]. Previous studies have demonstrated that this strain exhibits reduced nociceptive thresholds, reflected by shorter response latencies during both ictal and interictal periods, indicating increased sensitivity to thermal stimuli [8, 9]. Moreover, evidence suggests that this phenotype emerges early and remains stable across the lifespan, supporting the notion that altered nociceptive processing represents an intrinsic characteristic of the WAG/Rij model rather than a secondary consequence of recurrent seizures [4].

Pain processing is shaped not only by genetic predisposition but also by early-life environmental influences that critically regulate the development of neural systems involved in stress responsivity and sensory integration [10, 11, 12]. In rodents, maternal care—particularly tactile stimulation through licking and grooming—provides essential somatosensory input during sensitive developmental periods [13, 14]. Disruptions in early tactile experience, such as maternal deprivation or neonatal isolation, have been shown to induce long-lasting increases in pain sensitivity and alterations in nociceptive pathways [10, 11, 12]. These effects are mediated, at least in part, by enduring changes in stress-related systems, including the hypothalamic–pituitary–adrenal (HPA) axis, as well as modifications in nociceptive receptor expression and in central pain-modulatory circuits [12]. Conversely, enriched early-life tactile input has been reported to exert protective effects by optimizing stress responses and reducing vulnerability to maladaptive behavioral and sensory outcomes [13, 14].

In the context of WAG/Rij rats, early-life sensory experience may be particularly relevant, as dams of this strain exhibit impairments in maternal care, including reduced licking and grooming behaviors, likely associated with their depressive-like phenotype[13, 14]. Consequently, offspring may be exposed to diminished somatosensory input during critical periods of neurodevelopment. Previous studies have shown that interventions enhancing early-life environmental conditions, such as cross-fostering or tactile stimulation, can attenuate depressive-like behaviors and modulate epileptic activity in this model [13, 14]. However, despite the established link between early-life experience and neural development, it remains unclear whether such interventions can also influence long-term nociceptive processing in WAG/Rij rats.

Therefore, the present study aimed to investigate the long-term effects of neonatal tactile stimulation on thermal nociceptive responses in adult WAG/Rij rats. Specifically, we examined whether enhanced early-life somatosensory input modulates thermal nociceptive responses in a strain- and sex-dependent manner. In this way, the study seeks to clarify the contribution of early-life sensory experience to nociceptive processing in a genetic model of absence epilepsy and to provide insight into potential developmental mechanisms underlying pain-related comorbidity in this condition.

Materials and Methods

Animals and Experimental Design

All experimental procedures were approved by the Kocaeli University Animal Experiments Local Ethics Committee (KOU HADYEK 3/1–2023) and conducted in accordance with the guidelines for the care and use of laboratory animals (Ethical approval date: 29.03.2023). WAG/Rij and Wistar rats were obtained from the breeding colony of the Kocaeli University Experimental Medicine Research and Application Unit (DETAB), Türkiye. A total of 48 adult rats were included in the study, with 8 animals per group. Animals were randomly assigned to experimental groups. The groups consisted of: Wistar control males, Wistar control females, WAG/Rij control males, WAG/Rij control females, neonatal tactile stimulation (NTS)-applied WAG/Rij males, and NTS-applied WAG/Rij females. Animals were housed under standard laboratory conditions with a 12-h light/dark cycle (lights on at 7:00 a.m.) and had ad libitum access to food and water.

Following completion of the behavioral experiments, animals were maintained for use in subsequent experimental procedures approved under the same ethical protocol. At the end of all experimental procedures, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneal injection) and sacrificed by decapitation using a rodent guillotine. Depth of anesthesia was confirmed by the absence of the corneal reflex.

Rats were group-housed (four per cage) and allowed to acclimate to the laboratory environment prior to experimental procedures. Neonatal tactile stimulation was applied during the early postnatal period (postnatal days 8–14). During this period, pups were gently removed from the nest and subjected to tactile stimulation using a soft brush, administered three times daily at 09:00, 12:00, and 15:00. Each stimulation session lasted 15 minutes and was performed by the same experimenter to ensure procedural consistency [13, 15]. All behavioral assessments were conducted when the animals reached 12 months of age, ensuring evaluation during full adulthood. To minimize the effects of estrous cycle variations and fluctuations in sex hormones on behavioral outcomes, all behavioral experiments involving female rats were conducted with careful monitoring of the estrous cycle, ensuring that testing was performed during the same cycle phase [16]. The study was carried out between November 5, 2024 and December 26, 2025.

Hot Plate Test (Thermal Nociceptive Responses)

Thermal pain sensitivity was assessed using the hot plate test, a well-established stimulus-evoked method for evaluating pain-related behaviors in rodents [17, 18]. The test was performed using a hot plate apparatus (Commat Ltd., Ankara, Turkey), consisting of a heated metal surface with adjustable temperature control, enclosed within a transparent glass cylinder (20 cm in diameter, 25 cm in height) that restricted the animals' movement while allowing unobstructed observation. This assay measures

supraspinally integrated nocifensive responses, such as paw licking, shaking, or jumping, elicited by exposure to a noxious thermal stimulus [18].

In the present study, two noxious temperature conditions were employed to assess stimulus intensity–dependent differences in thermal nociceptive processing. Both 54.0 ± 0.5°C and 52.0 ± 0.5°C represent externally applied thermal stimuli; however, differences in stimulus intensity are known to influence behavioral responses. The higher temperature (54.0 ± 0.5°C), commonly used in the hot plate test, elicits rapid nocifensive reactions and primarily reflects acute stimulus-driven nociceptive responses [19]. In contrast, the lower temperature (52.0 ± 0.5°C) provides greater sensitivity for detecting subtle alterations in thermal nociceptive processing and has been used in studies examining enhanced thermal responsiveness under conditions associated with neuropathic-like pain [19, 20].

Animals were individually placed on the heated surface, and the latency to the first nocifensive response (paw licking, flicking, or jumping) was recorded. A 50-second cutoff was applied to prevent tissue damage. Shorter latencies were interpreted as increased thermal sensitivity (hyperalgesia), whereas prolonged latencies indicated reduced nociceptive responsiveness. Thus, the use of two temperature settings allowed comparison of responses to high-intensity thermal stimulation with those obtained under lower noxious conditions, which may be more sensitive for detecting alterations in nociceptive processing associated with mechanisms such as central sensitization and neuroinflammatory changes [21, 22].

Locomotor Activity Test

Locomotor activity was also assessed using a rat activity monitoring system (May AHP 0603, Commat Ltd., Ankara, Turkey), consisting of a Plexiglas test chamber, a computer interface, and dedicated analysis software. Animals were transferred to the testing room and allowed to acclimate for 1 hour prior to the assessment. Each rat was then placed individually into a Plexiglas chamber (42 × 42 × 30 cm) equipped with infrared photocell sensors. The system included 15 pairs of infrared beams and detectors arranged at 2.5 cm intervals along the horizontal plane and 4.5 cm intervals along the vertical plane. Locomotor activity was continuously recorded over a 10-minute session and subsequently analyzed. Total distance traveled was used as an index of locomotion [13].

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 10.2.3 (GraphPad Software, USA). Data are presented as mean ± SEM. The normality of data distribution was assessed using the Kolmogorov–Smirnov test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test was applied. A p-value < 0.05 was considered statistically significant.

Results

Thermal nociceptive responses assessed using the hot plate test at 54 ± 0.5°C revealed no significant differences among any of the experimental groups in either gender.

One-way ANOVA showed no significant group effect ($p > 0.05$) among the female rat groups. Tukey's post hoc comparisons confirmed the absence of significant differences between all sub-group comparisons, e.g. Wistar and WAG/Rij rats (mean difference = -0.50, 95% CI [-3.76, 2.76], $p > 0.05$), Wistar and NTS-treated WAG/Rij rats (mean difference = -0.38, 95% CI [-3.63, 2.88], $p > 0.05$), and WAG/Rij and NTS-treated WAG/Rij rats (mean difference = 0.13, 95% CI [-3.13, 3.38], $p > 0.05$). The group mean response latencies were 9.88 ± 0.83 s in Wistar controls, 10.38 ± 0.98 s in WAG/Rij controls, and 10.25 ± 0.92 s in NTS-treated WAG/Rij rats (see Fig. 1).

Similarly, in males, one-way ANOVA revealed no significant group effect ($p > 0.05$). Tukey's post hoc analysis showed no significant differences between subgroups, e.g., Wistar and WAG/Rij rats (mean difference = -2.13, $p > 0.05$), Wistar and NTS-treated WAG/Rij rats (mean difference = -1.63, $p > 0.05$), or WAG/Rij and NTS-treated WAG/Rij rats (mean difference = 0.50, $p > 0.05$). The group mean response latencies were 8.63 ± 0.60 s in Wistar controls, 10.75 ± 0.96 s in WAG/Rij controls, and 10.25 ± 1.07 s in NTS-treated WAG/Rij rats (see Fig. 2).

At 52 ± 0.5°C, significant differences in pain response latency were observed. In females, one-way ANOVA revealed a significant group effect ($p < 0.05$). Tukey's post hoc comparisons showed that WAG/Rij rats had significantly shorter latencies compared to Wistar controls (mean difference = 5.38, 95% CI [1.08, 9.42], $p < 0.05$). Similarly, NTS-treated WAG/Rij rats differed significantly from Wistar controls (mean difference = 7.75, 95% CI [3.88, 11.62], $p < 0.001$). No significant difference was observed between WAG/Rij and NTS-treated WAG/Rij rats (mean difference = 2.38, $p > 0.05$). Mean latencies were 22.38 ± 1.45 s in Wistar controls, 17.00 ± 0.76 s in WAG/Rij controls, and 14.63 ± 0.92 s in NTS-treated WAG/Rij rats (see Fig. 3).

In males, one-way ANOVA revealed a significant group effect ($p < 0.001$). Tukey's post hoc analysis showed that WAG/Rij rats exhibited significantly shorter latencies compared to Wistar controls (mean difference = 9.25, 95% CI [0.68, 17.82], $p < 0.05$). In contrast, NTS-treated WAG/Rij rats showed significantly longer latencies compared to WAG/Rij controls (mean difference = 17.50, 95% CI [8.26, 26.90], $p = 0.001$). No significant difference was observed between Wistar and NTS-treated WAG/Rij rats (mean difference = -8.25, $p > 0.05$). Mean latencies were 22.50 ± 2.69 s in Wistar controls, 13.25 ± 0.59 s in WAG/Rij controls, and 30.75 ± 3.12 s in NTS-treated WAG/Rij rats (see Fig. 4).

Locomotor activity analysis revealed no significant differences among experimental groups in either gender. In females, total distance traveled was 404.9 ± 38.73 cm in Wistar controls, 337.0 ± 32.70 cm in WAG/Rij controls, and 369.1 ± 32.77 cm in NTS-treated WAG/Rij rats. One-way ANOVA showed no significant group effect ($p > 0.05$), and Tukey's post hoc comparisons confirmed the absence of significant differences between groups (all $p > 0.05$) (see Fig. 5).

In males, locomotor activity was 313.9 ± 19.98 cm in Wistar controls, 337.1 ± 29.17 cm in WAG/Rij controls, and 325.8 ± 36.68 cm in NTS-treated WAG/Rij rats. One-way ANOVA revealed no significant group effect ($p > 0.05$), with no significant differences detected in post hoc comparisons (all $p > 0.05$) (see Fig. 6).

Discussion

The present study demonstrates a stimulus intensity– and sex-dependent pattern of thermal nociceptive responses in WAG/Rij rats. Thermal nociceptive responses to high-intensity heat stimulation (54 ± 0.5°C) did not differ between strains or sexes and were not affected by NTS. In contrast, under lower-intensity heat stimulation (52 ± 0.5°C), WAG/Rij rats exhibited enhanced thermal responsiveness compared to Wistar controls. Notably, NTS produced a sex-dependent effect, normalizing thermal nociceptive responses in adult male WAG/Rij rats, while no significant effect was observed in females. The absence of differences in locomotor activity indicates that these findings reflect specific alterations in nociceptive processing rather than general behavioral changes.

The lack of group differences at 54 ± 0.5°C is consistent with evidence that higher stimulus intensities reduce the sensitivity of nociceptive assays and may mask subtle differences between experimental groups [23, 24, 25]. Under such conditions, nociceptive responses are rapidly triggered by high-threshold peripheral nociceptors, leading to relatively uniform behavioral outputs across groups [26, 27].

In contrast, significant differences emerged at 52°± 0.5°C, a lower stimulus intensity that provides greater sensitivity for detecting alterations in nociceptive processing [23, 24].The selective emergence of group differences under these conditions suggests that both the WAG/Rij phenotype and the effects of NTS are more apparent under lower-intensity stimulation conditions rather than at higher stimulus intensities

The enhanced thermal nociceptive responses observed in WAG/Rij rats are consistent with previous studies reporting reduced nociceptive thresholds in this strain [8, 9]. This phenotype appears to be intrinsic and stable, suggesting that altered nociceptive processing is not merely a consequence of seizure activity [4, 7]. A likely contributing mechanism is neuronal hyperexcitability, a shared feature of both epilepsy and chronic pain, which facilitates amplified sensory responses and contributes to central sensitization [4, 22]. In addition, alterations in neuroinflammatory and epigenetic processes have been implicated in this model and may further shape nociceptive processing [4]. At the systems level, dysfunction within corticothalamocortical circuits, a hallmark of absence epilepsy, may also contribute to altered sensory processing [4, 5, 7].

Importantly, NTS selectively modulated thermal nociceptive responses in male WAG/Rij rats, normalizing response latencies toward control levels. This finding supports the view that early-life somatosensory experiences influence the development of nociceptive systems. Previous studies have shown that tactile stimulation reduces vulnerability to stress-induced hyperalgesia and promotes adaptive regulation of nociceptive pathways, partly through long-term modulation of stress-related systems such as the

hypothalamic–pituitary–adrenal axis [10, 28, 29, 30]. In addition, early sensory experience may shape structural and functional plasticity in somatosensory circuits, contributing to long-term changes in nociceptive processing [31, 32, 33]. Given the reduced maternal care observed in WAG/Rij dams [14], NTS may act as a compensatory source of tactile input, thereby mitigating the development of enhanced nociceptive responsiveness in adulthood [13, 14].

The absence of a comparable effect in females suggests a sex-dependent limitation in the efficacy of early-life tactile interventions. Sex differences in nociceptive processing involve distinct neurobiological mechanisms, including hormone-dependent modulation and differential neuroimmune signaling [30, 34, 35, 36]. Consistent with this, the long-term effects of early-life interventions such as neonatal handling have been shown to depend on androgen-mediated mechanisms and are typically more pronounced in males [29].

The locomotor activity findings further support the specificity of the observed effects, as no group differences were detected. This indicates that the observed changes in thermal nociceptive responses are unlikely to be attributable to alterations in general activity levels or motor function [37, 38].

Several limitations should be considered. First, thermal nociceptive responses were assessed using a single behavioral paradigm, which does not capture all dimensions of nociception. Second, although estrous cycle phase was controlled, hormonal influences may still have contributed to variability in females. Third, the absence of direct neurobiological measurements limits mechanistic interpretation. In addition, maternal behavior was not directly quantified, and the use of a single genetic model may limit generalizability. Future studies incorporating multimodal and mechanistic approaches are needed to further elucidate these processes.

Overall, these findings indicate that early-life tactile stimulation exerts long-term, sex-dependent effects on thermal nociceptive responses under lower-intensity heat stimulation in WAG/Rij rats, likely through modulation of mechanisms underlying altered nociceptive processing rather than high-intensity stimulus-driven responses.

Conclusions

The findings show that responses to thermal stimulation in WAG/Rij rats vary according to stimulus intensity and are shaped by early-life experience in a sex-dependent manner. Responses to higher temperature stimulation were similar across groups, whereas lower temperature stimulation revealed enhanced responsiveness in WAG/Rij rats, indicating that differences in nociceptive processing become more detectable under conditions that allow finer discrimination of behavioral responses.

Compared with Wistar controls, untreated WAG/Rij rats displayed greater responsiveness to lower-intensity thermal stimulation. Early-life tactile stimulation reduced this enhanced responsiveness in male WAG/Rij rats but had no significant effect in females, suggesting a sex-dependent influence of early sensory experience on the development of nociceptive systems. The absence of differences in

locomotor activity supports the interpretation that these effects are specific to nociceptive processing rather than general behavioral changes.

Overall, these results highlight the importance of early-life somatosensory input in shaping nociceptive regulation in genetically susceptible populations and underscore sex as a critical factor influencing the outcomes of early-life interventions.

Declarations

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Consent for publication

Not Applicable

Ethics Approval Statement

All experimental procedures were approved by the Kocaeli University Animal Experiments Local Ethics Committee (KOU HADYEK 3/1–2023) and conducted in accordance with the guidelines for the care and use of laboratory animals.

Declaration of Interests

The authors have no conflicts of interest to declare.

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Author Contributions

Concept – A.B., G.İ.; Design – A.B., G.İ.; Supervision – G.İ., G.G., T.M.B.; Resources – G.İ., A.B.; Materials – A.B., G.İ., V.K.G.; Data Collection and/or Processing – A.B., G.İ., T.M.B., V.K.G.; Analysis and/or Interpretation – G.İ., G.G., A.B, T.M.B.; Literature Search – A.B., G.İ., V.K.G.; Writing Manuscript – A.B., G.İ., T.M.B., G.G.; V.K.G.; Critical Review – A.B., G.İ., T.M.B., G.G.

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Figures

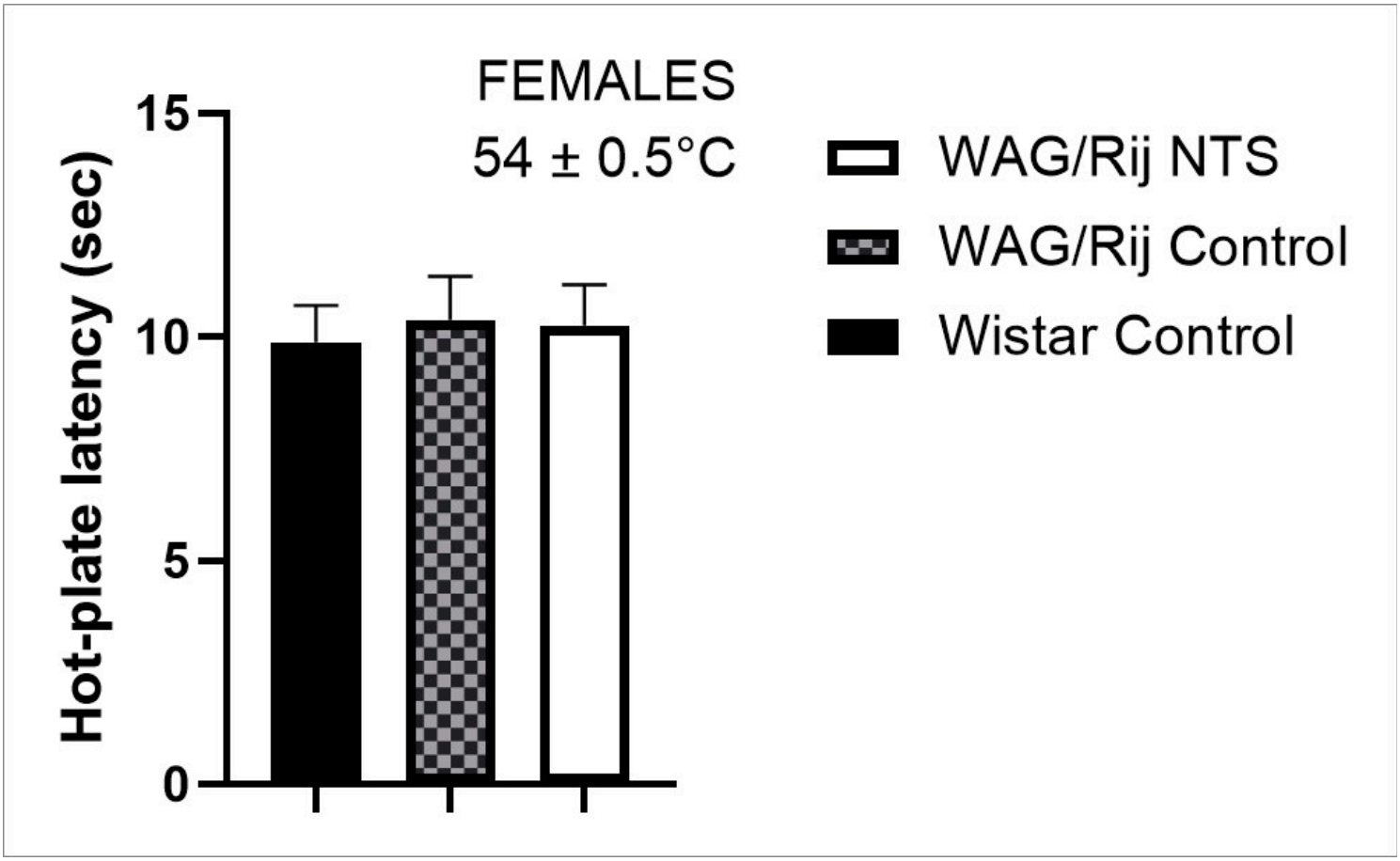


Figure 1

Thermal nociceptive responses in female rats assessed by the hot plate test at 54 ± 0.5°C. Data are presented as mean ± SEM (n = 8).

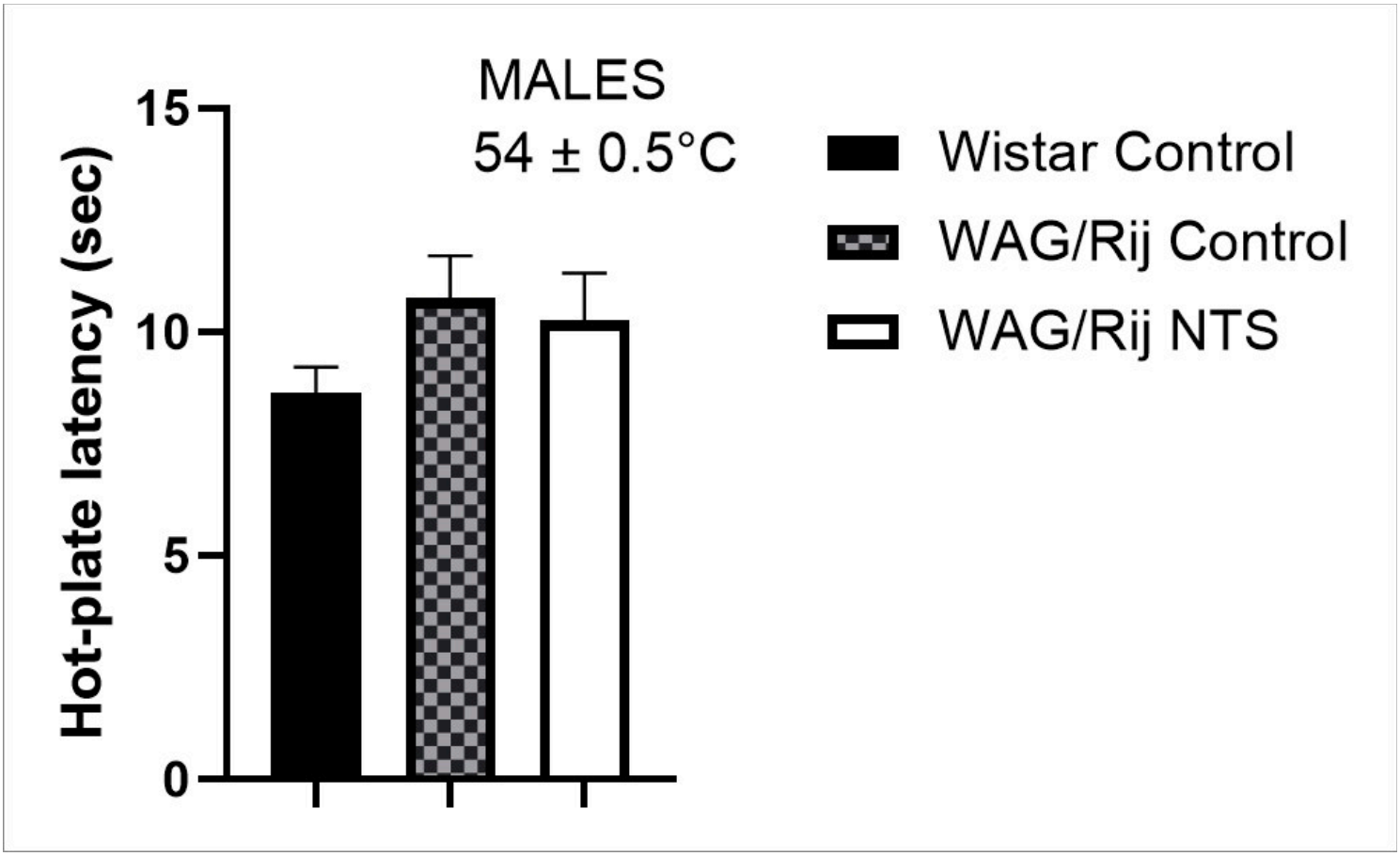


Figure 2

Thermal nociceptive responses in male rats assessed by the hot plate test at 54 ± 0.5°C. Data are presented as mean ± SEM (n = 8).

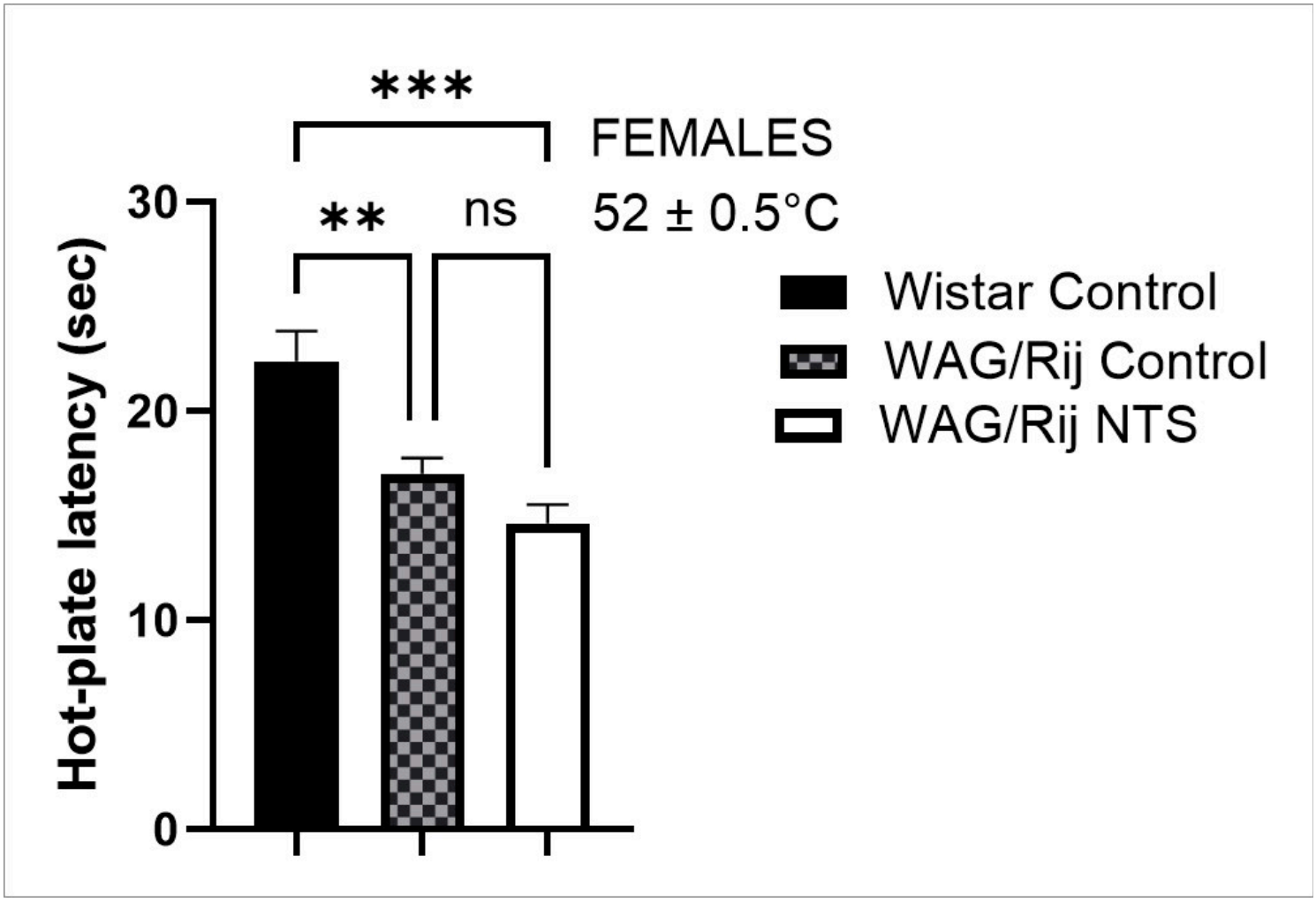


Figure 3

Thermal nociceptive responses assessed using the hot plate test at 52.5 ± 0.5°C in female rats. Data are presented as mean ± SEM (n = 8).

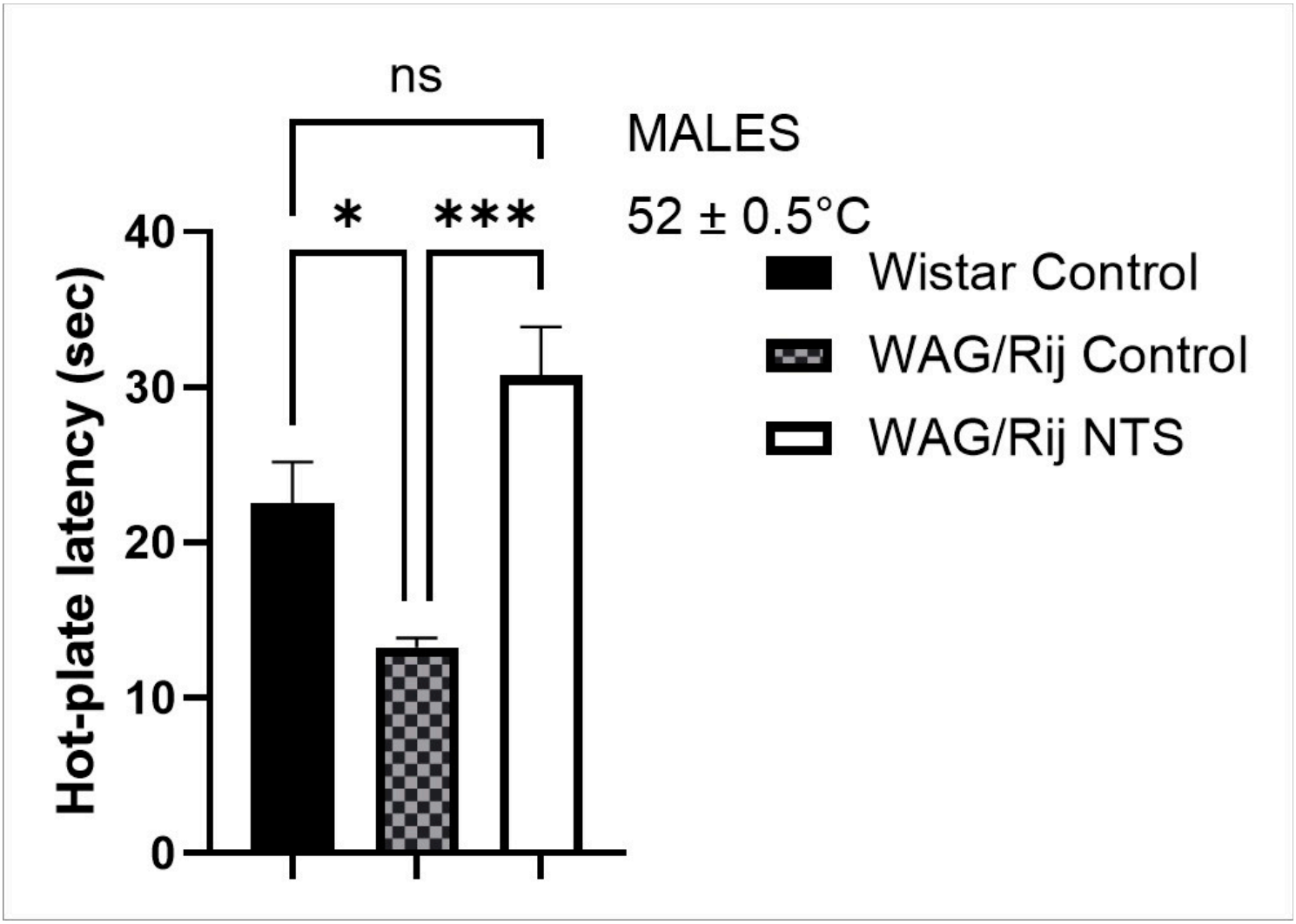


Figure 4

Thermal nociceptive responses assessed using the hot plate test at 52.5 ± 0.5°C in male rats. Data are presented as mean ± SEM (n = 8).

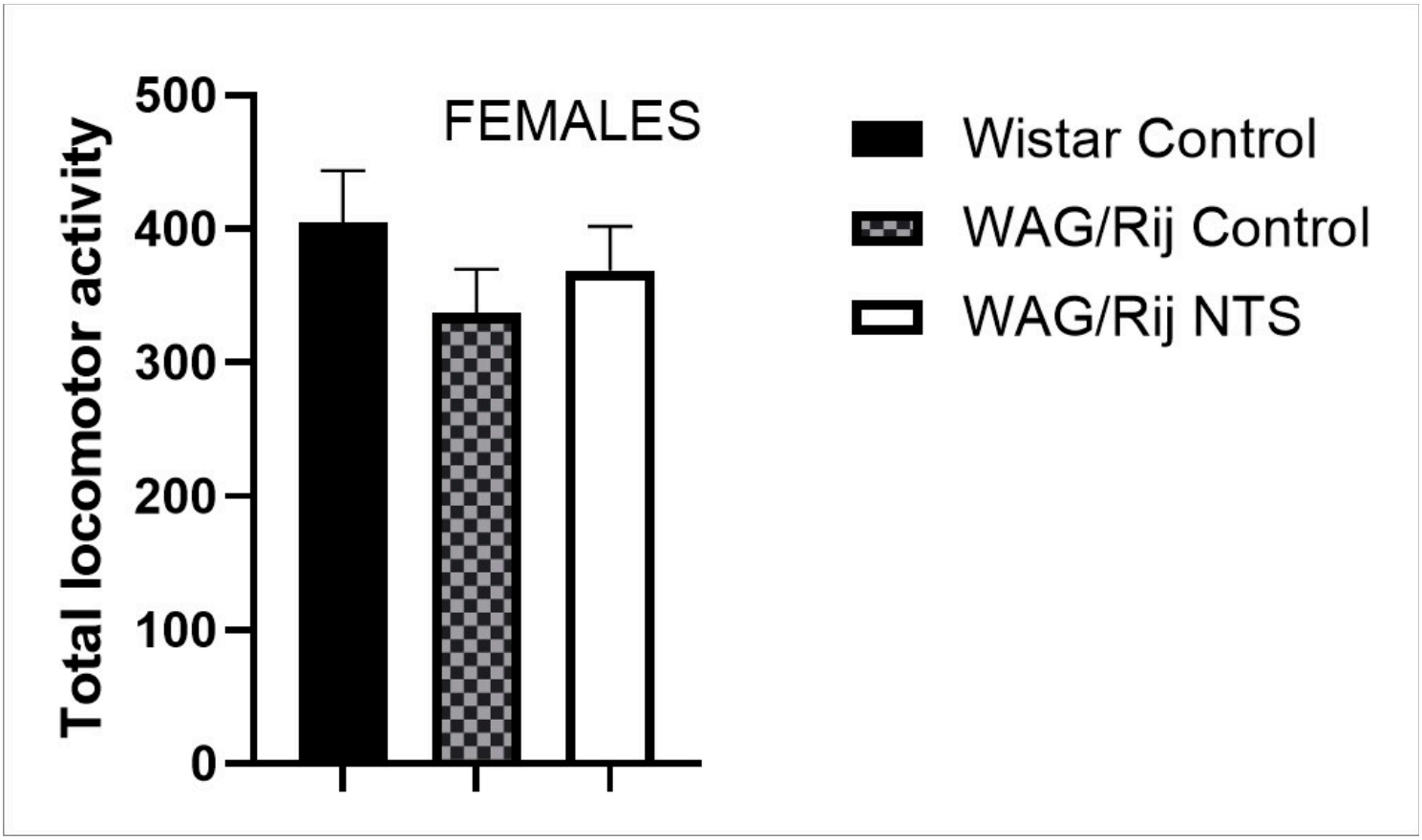


Figure 5

Total locomotor activity in female rats. Data are presented as mean ± SEM (n = 8).

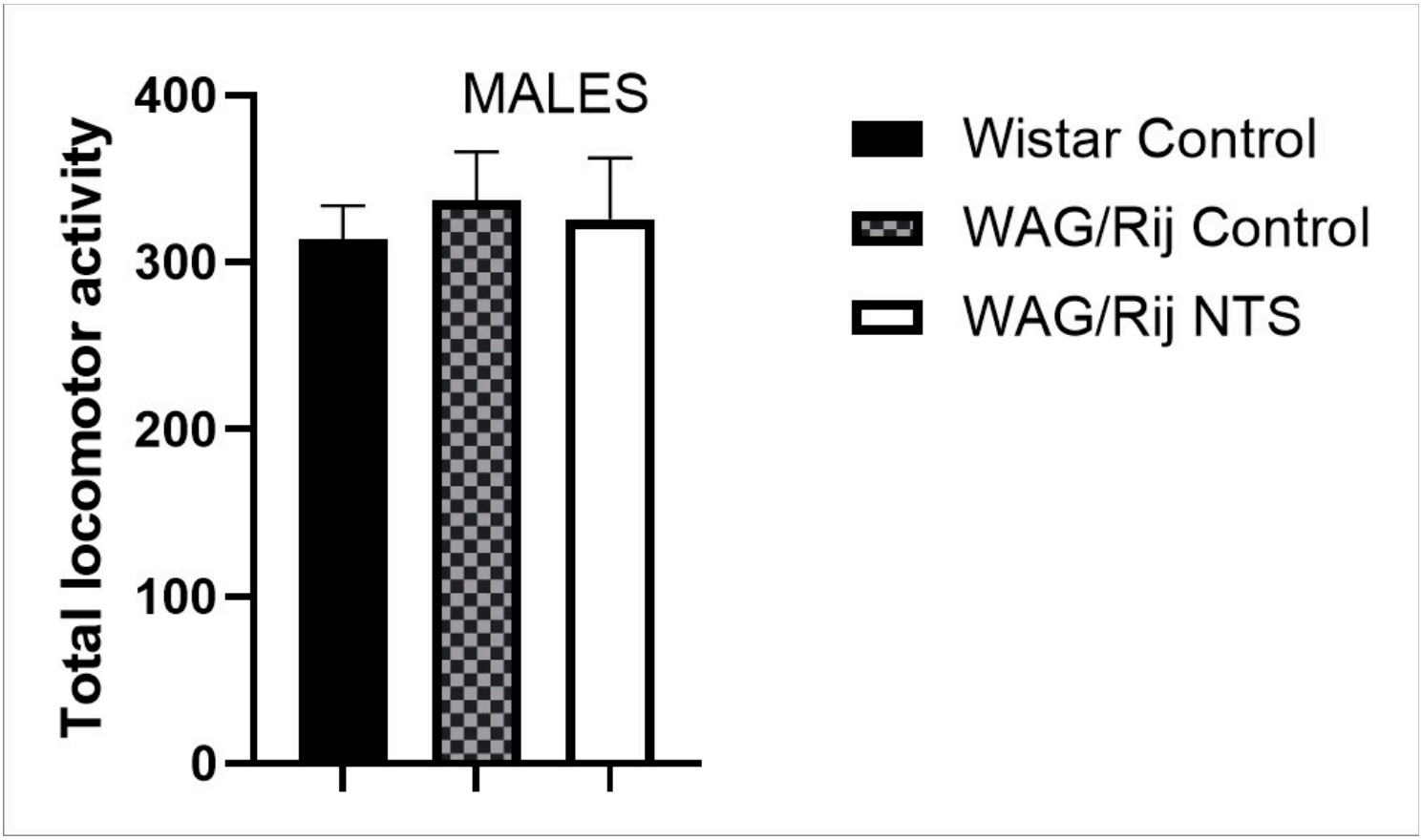


Figure 6

Total locomotor activity in male rats. Data are presented as mean ± SEM (n = 8).