HHS Public Access

Author manuscript

Behav Brain Res. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Behav Brain Res. 2016 January 1; 296: 326–330. doi:10.1016/j.bbr.2015.09.028.

Voluntary Exercise Facilitates Pair-Bonding in Male Prairie Voles

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Abstract

The neuropeptides oxytocin and vasopressin have been implicated in exercise, as well as monogamy and parental behavior. In this study, we compared behavioral and neuroendocrine effects of access to an exercise wheel versus the sedentary state typical in lab animal housing. Male prairie voles (*Microtus ochrogaster*) were studied because of their extensive repertoire of social behaviors including pair bond formation and biparental care, which are influenced by oxytocin and vasopressin. Subjects in one group had access to a running wheel in their cage (Wheel), and voluntarily ran approximately 1.5 km/day for six weeks; these animals were compared to males in standard housing conditions (n = 10 / group). Males allowed to exercise formed partner preferences significantly faster than controls and exhibited fewer oxytocin neurons, as measured by immunohistochemistry in the bed nucleus of the stria terminalis. We observed no differences in terms of anxiety-related behavior, or alloparental responsiveness. Males with a running wheel equipped cage gained more total body weight, and by the end of the six weeks were found to have less subcutaneous fat and larger testes as a percentage of bodyweight. The changes to gonadal regulation and pair-bonding behavior associated with voluntary exercise are discussed in terms of their possible relevance to the natural history of this species.

Keywords

Pair bonding; Exercise; Oxytocin; Vasopressin; Testes; Prairie vole

Introduction

Regular voluntary exercise is one of the best-established mechanisms for increasing wellness and behavioral plasticity [1]. Social bonds and other positive relationships also are protective, especially in the face of stressful experiences [2]. The neuropeptides oxytocin (OT) and arginine vasopressin (AVP) have been shown to have a central role in social bonding [3], and parental behavior [4], as well as adaptation to various stressors [5]. OT in

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particular has been shown to be one of the mechanisms by which social support infers stress resilience [6, 7]. Likewise, voluntary exercise produces anxiolytic effects [1, 8] and also leads to the up-regulation of OT systems [9, 10].

OT plays important roles in the autonomic changes that follow access to voluntary exercise. In exercise trained animals, OT acts in the nucleus tractus solitarius (NTS) to restrain exercise-induced tachycardia [11]. Furthermore, exercise training in rats leads to increased levels of OT in the NTS [9]. OT increases parasympathetic cardiac tone to slow the heart by acting on the dorsal vagal complex (including the NTS and the dorsal motor nucleus of the vagus (DMX)) [12].

Social behaviors, including alloparental care [4, 13] and pair-bond formation [3] also are regulated by the simultaneous actions of both OT and AVP. In male prairie voles, the expression of alloparental care also involves a sustained increase in heart rate, similar to an acute bout of exercise [14-16]. Exercise increases the expression and release of these neuropeptides, and social support is likewise anxiolytic. In this context, we examined the hypothesis that male voles allowed to voluntarily exercise would be more likely to express alloparental care, form pair-bonds and explore a novel open-field (used to index anxiety). At the conclusion of the study, we examined the effects of exercise on fat deposition, and testes weight, as well as the central abundance of cells and fibers immunoreactive to OT and AVP.

Methods

Subjects

Male F2 or F3 descendants of wild prairie voles caught near Champaign, Illinois were used in these experiments at 60-90 days of age. Subjects were maintained on a 14/10 hour light/dark cycle in a temperature and humidity controlled vivarium. Food (Purina rabbit chow) and water were available *ad libitum*. All test subjects were sexually naïve and had never been exposed to pups. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Illinois at Chicago Institutional Animal Care and Use Committee.

Experimental Design

Male vole subjects were randomly assigned to either of two conditions for 6 weeks: placed with their sibling in a standard mouse cage (18 × 28 × 20 cm) equipped with a running wheel (Wheel, n = 10), or left with their same-sex sibling in a similar cage without a running wheel (Sedentary, n = 10). Animals of each condition were weighed once every week. In the Exercise treatment, distance ran was calculated via the equipment's tally of wheel rotations which were recorded every day and halved to account for the two animals in each cage. Pair housing was used to avoid the effects of social isolation, to which voles are particularly sensitive [17].

Following 6 weeks in these housing conditions, animals were subjected to three behavioral tests in order of presumed increasing salience. Experiments began during the lights-on period between 10:00 and 11:00 AM. Subjects were exposed to an Open Field Test (OFT), then returned to their home cage for 24 hours, then tested in an Alloparental Test, returned

to their home cage for 24 hours, and finally cohabitated with a novel female for 30 minutes, followed immediately by a Partner Preference Test (PPT) designed to assess the tendency to prefer a familiar female versus one that is otherwise similar but unfamiliar. Behavior was later analyzed by two trained, experimentally-blind observers using Noldus Observer (Noldus Information Technology, The Netherlands).

Open Field Test

The open field consisted of a clear plexiglas testing arena 42 × 42 cm square, with walls 30 cm high, under indirect lighting at typical colony levels. Testing in the arena lasted for 10 minutes, during which time behavior was video recorded. Observers recorded the amount of time spent in the center quarter of the arena, time spent autogrooming and the amount of total locomotor activity. Time spent in the center of the arena is often used as an index of both state and trait anxiety [18].

Alloparental Test

Testing of alloparental responses to an unfamiliar 1-3 day old pup was conducted based on previously published procedures [4]. Testing occurred in a novel cage in which a male was immediately presented with a pup and behavior recorded for 20 minutes. In the rare instances of an adult male expressing aggression towards the pup, the test was aborted and the pup either returned to its home cage or euthanized. Observers recorded the latency to approach the pup and durations of: carrying the pup, licking/grooming the pup, huddling over the pup and total time in contact with the pup.

Partner Preference Test

Testing of the tendency to form a partner preference was conducted based on previously published procedures [4]. Males were allowed to cohabitate for 30 minutes with age- and weight matched females that had had no previous reproductive experience and were not receptive to mating attempts. The cohabitation time required to induce a partner preference varies among studies, but 30 minutes was selected as it is generally insufficient time to form a selective preference. Immediately following cohabitation, stimulus females (henceforth referred to as Partners) were removed and tethered in place in a separate testing cage. Another age and weight matched female, previously unfamiliar to the male (henceforth referred to as a Stranger), was tethered in a separate but adjoining cage and male subjects placed into a neutral cage with free access to both Partner and Stranger for 3 hours. During preference tests, social preferences were determined as a function of time spent in side by side contact with a familiar versus an unfamiliar female.

Tissue Collection

Twenty-four hours after the final behavioral test, animals were sacrificed and tissue collected and brains processed for immunohistochemistry, according to previously published procedures [4, 16]. The number of cells in the medial region of the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus of the hypothalamus (PVN), along with neuronal projections within the DMX, NTS and nucleus ambiguus (NA) staining for

immunoreactivity for OT and AVP were quantified by an individual blind to the history of the subjects.

Consistent with IACUC policy, subjects immediately were anesthetized with a mixture of ketamine (67.7 mg/kg; NLS Animal Health, Owings Mills, MD) and xylazine (13.3 mg/kg; University of Illinois Hospital Pharmacy, Chicago, IL) administered intraperitoneally and then quickly euthanized via cervical dislocation. Final body weights were recorded along with testes and subcutaneous fat.

Statistical Analysis

Data are presented as means ± standard errors in all text and figures. All analyses were conducted using SPSS 19 (SPSS inc., USA), with α set at 0.05. Subject bodyweights were compared via a repeated measures ANOVA; when a significant time by treatment interaction was found, a post-hoc t-test was conducted on each group to examine the differences over time. Testes weight and subcutaneous fat were both calculated as percentage of bodyweight and compared via two tailed student's t-test, as were behaviors in the OFT, Alloparental Test, PPT, OT-ir and AVP-ir. Data were log transformed when found to be non-normal or heteroschedastic.

Results

Subjects in the Wheel condition ran an average of 1.5 km/day/animal over the course of the 6 weeks of the experiment (Fig. 1A). Body weight was influenced by time [$F(5,14) = 19.661$, $p < 0.001$] as well as a time by condition interaction [$F(5,14) = 5.245$, $p = 0.006$]; posthoc t-tests revealed significantly greater weight in the Wheel condition over the final 3 weeks of observation ($p \leq 0.03$ for all comparisons) (Fig. 1B). Males from the Wheel condition had larger testes (1.8 ± 0.2% vs. 1.2 ± 0.1%, $p = 0.023$) and less body fat (0.7 ± 0% vs. 1.4 ± 0.1%, $p < 0.001$) (Fig. 1C). There were no differences in terms of time spent in the center of the OFT, grooming, or total locomotor activity ($p > 0.05$ for all comparisons) or between treatments in the tendency to show alloparental behavior. With the exception of a single animal in the Sedentary condition, all subjects responded alloparentally to a pup.

Males in the Wheel condition showed a significant preference for the familiar Partner over the novel Stranger (1472.8 ± 527.5 vs. 20.3 ± 19.7 seconds, $p = 0.024$), while males in the Sedentary condition showed no preference for the Partner vs. the Stranger (797.5 ± 393.5 vs. 629.4 ± 415.8 seconds $p = 0.8$) (Fig. 2B).

Figure 3 illustrates the results of immunohistochemical staining for OT-ir in the BNST , where Sedentary males were found to have more OT neurons (12.9 ± 2.0 vs. 6.4 ± 1.3, $p = 0.007$). OT-ir in the PVN or brainstem autonomic regions (DMX, NA and NTS) did not differ by groups ($p > 0.05$ for all comparisons). Significant group differences in AVP-ir were not detected ($p > 0.05$ for all comparisons). In general, OT-ir staining was more readily visible in comparison to AVP-ir, for which staining was too light for analysis outside the PVN.

Discussion

Male prairie voles engaged in voluntary exercise when given access to exercise wheels, running approximately 1.5 km / day. The findings of the present study illustrated the capacity of voluntary exercise to facilitate the formation of a partner preference pair bonding, along with an increase in body weight and testes size and a reduction of OT in the BNST.

Bodyweight increased over the course of the 6 weeks in both groups, with the largest changes in bodyweight being measured in the males with access to a running wheel. Males with access to the exercise wheel also had larger tests and less body fat. The size of testes in the Sedentary group, studied under a 14/10 light/dark cycle, corresponded to those of prairie voles living in conditions of 16 hours of light / day [19]. The data from the present study are in contrast to results from an earlier study in meadow voles, a non-monogamous species. In meadow voles tested under long day conditions, 8 weeks of exercise produced *decreased* bodyweight, with no effect on testes size; however, when tested under short day conditions, meadow voles showed an exercise-related increase in testes size [20]. The physiological mechanisms for these apparent species differences in the response to environmental cues remain to be discovered.

In the present study, exercise did not significantly affect the tendency toward alloparental behavior in prairie voles. However, consistent with previous studies [4, 14], the majority of prairie voles tested here responded parentally towards the pup (one animal in the Sedentary treatment attacked the pup). Based on previous research, the high levels of alloparental behavior in this species do not differ between males with and without reproductive experience [15]. Thus, detecting effects of exercise may have been occluded by a ceiling effect.

The induction of pair bond formation in this species can be influenced by both OT and AVP [3], and we hypothesized that changes in one or both of these peptides would follow exercise experience. Exercise training produces a lower resting heart rate as well as a diminished tachycardic response to future exercise, both of which involve the actions of OT [9, 10]. Exercise also increases the density of OT projections to the NTS [21] and DMX [9, 11]. The absence of changes in OT in the NTS/DMX in the present study could be due to a shorter period of time spent with access to voluntary exercise (6 versus 12+ weeks). However, in the present study exercise was associated with fewer OT immunoreactive neurons in the BNST, which could suggest either diminished synthesis or depletion via release prior to sacrifice. Exercise adaptation in rodents has produced mixed results in tests of anxiety [22-24], although it is largely beneficial in humans [8]. In the present study we did not see a significant effect on behavior in the OFT in male voles. These findings suggest the need for a more in-depth analysis of the role of voluntary exercise in endocrine regulation, including modulation of activity in the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary gonadal (HPG) axes. Future efforts should also include locked wheel cages as a more precise control condition.

Male prairie voles given access to regular exercise formed partner preferences faster than their sedentary counterparts, possibly indicative of an increased readiness to pair-bond. We had predicted that this difference would be associated with an increase in OT and/or AVP. However, the only change in these peptides detected in the present study was an exercise-related decrease in OT in the BNST. Thus, data from the present study do not support the hypothesis that this is a simple matter of increased synthesis of OT or AVP, although changes to expression of receptors for these peptides remains to be explored.

Prairie voles can experience reproductive suppression when living with close kin [25]. Access to a running wheel may have induced physiological changes related to those seen when males leave the natal nest and begin to roam in search of a mate and/or a home territory, which could lead to a disinhibition of the HPG axis. An exercise-induced increase in testes size might also translate to an increased preference among females for such males [26]. The results of the present study suggest and interconnectedness of adaptations associated with physical activity, the endocrinology of the HPG and HPA axes, and the capacity to form partner preferences. However, at present the relationship between exercise, male reproductive status, sex hormones, neuropeptides and pair bond formation is not fully understood.

Acknowledgements

This work was made possible by the efforts of Gessa Suboc and Lisa Sanzenbacher, as well as the animal care staff at the University of Illinois at Chicago, and also by the support of the NIH: MH72935 (CSC), HD38490 and HD075750.

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Highlights

- Male prairie voles were given access to running wheel equipped cages
- Exercised males gained more weight, had less subcutaneous fat, and larger testes
- Exercised males formed partner preferences faster than control males

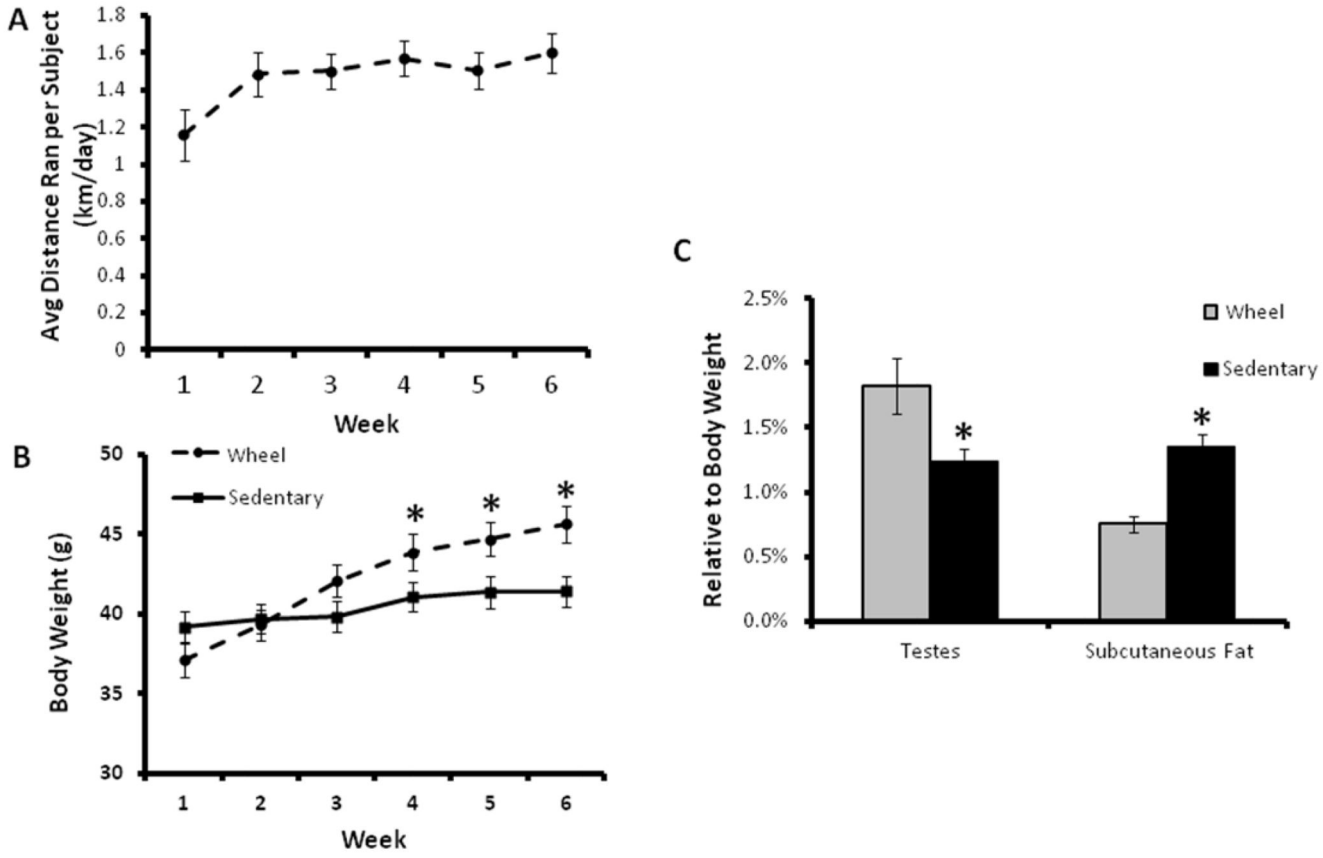

Figure 1.

(A) Average distance run per day per animal over the course of 6 weeks in the Exercise treatment. (B) Average body weight over the course of 6 weeks in each treatment, weight increased over time in the Exercise treatment only (n = 10 / group, $p < 0.05$). (C) Testes and subcutaneous fat weight as a percentage of body weight. Testes were significantly larger in the Exercise treatments and subcutaneous fat was significantly smaller ($p < 0.05$ for all comparisons). * indicates significant difference between Sedentary and Exercise conditions.

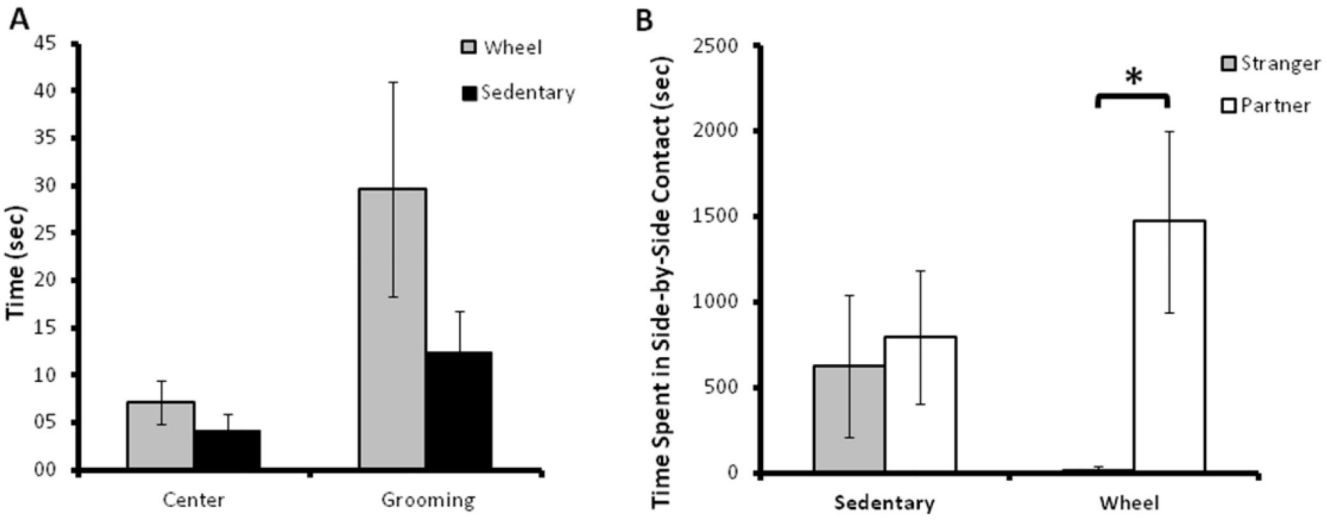

Figure 2.

Behavioral effects of the Exercise relative to Sedentary animals (n = 10 / group). (A) Males in the Exercise and Sedentary conditions were not significantly different in either time spent in the center of the open field or grooming ($p > 0.05$). (B) Males in the Exercise condition formed significant partner preferences after 30 minutes of cohabitation with a novel female (* denotes $p < 0.05$) while Sedentary males did not.

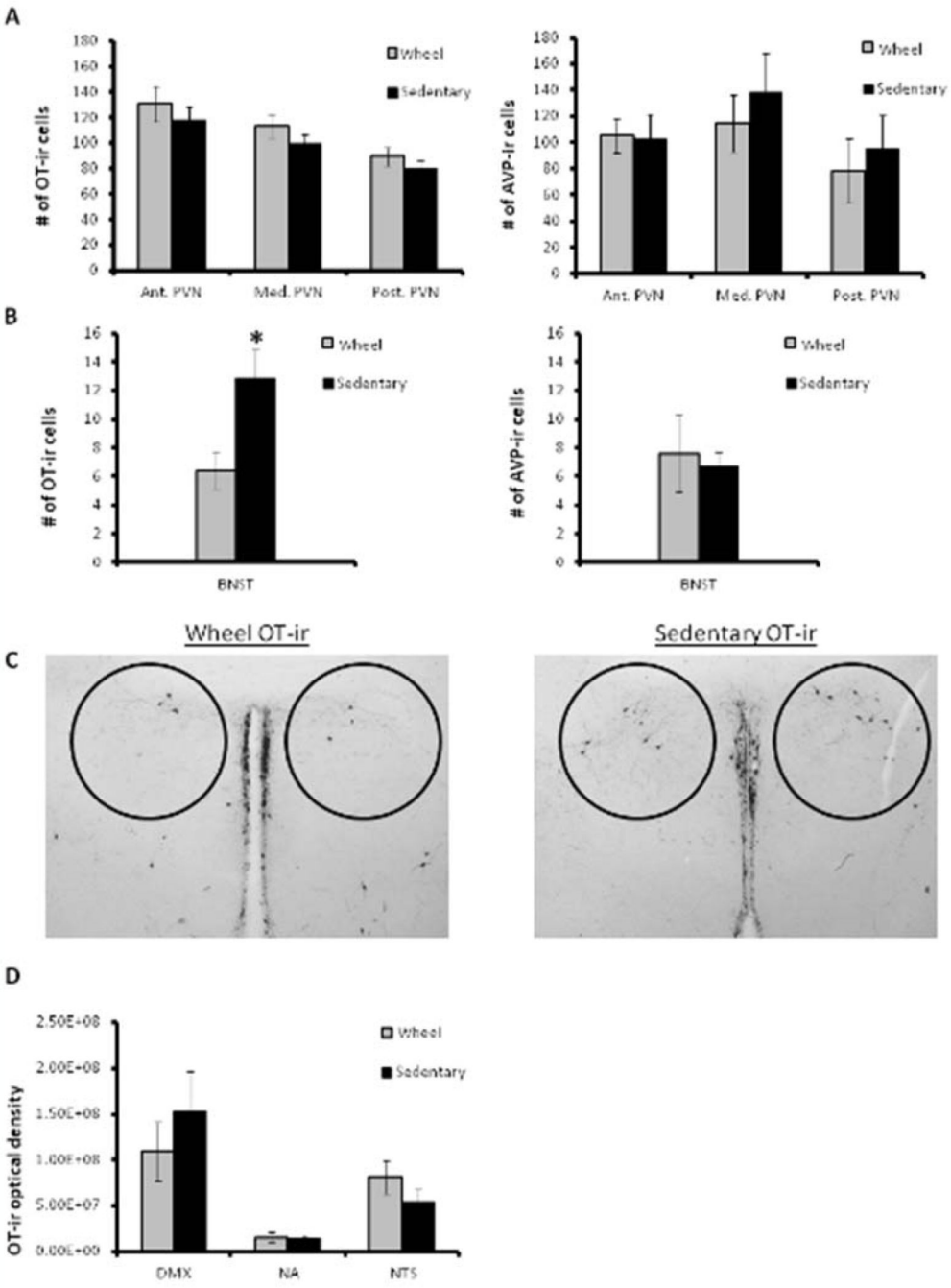

Figure 3.

Immunohistochemical results for OT staining in various brain regions (n = 10 / group). (A) There were no significant effects in any of the three sections of the PVN for either OT or AVP. (B) Males in the Sedentary condition had significantly more OT neurons in the BNST than the Exercise conditions (* denotes $p < 0.05$). (C) Representative photomicrographs of the BNST taken at 4× magnification for OT immunoreactivity. (D) There were no significant effects on OT immunoreactivity in any of the brainstem regions examined.