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Effects of intrinsic aerobic capacity and ovariectomy on voluntary wheel running and nucleus accumbens dopamine receptor gene expression

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Abstract

Rats selectively bred for high (HCR) and low (LCR) aerobic capacity show a stark divergence in wheel running behavior, which may be associated with dopamine (DA) system in the brain. HCR possess greater motivation for voluntary running along with greater brain DA activity compared to LCR. We recently demonstrated that HCR are not immune to ovariectomy (OVX)-associated reductions in spontaneous cage (i.e. locomotor) activity. Whether HCR and LCR rats differ in their OVX-mediated voluntary wheel running response is unknown.

PURPOSE—To determine whether HCR are protected from OVX-associated reduction in voluntary wheel running.

METHODS—Forty female HCR and LCR rats (age ~27 weeks) had either SHM or OVX operations, and given access to a running wheel for 11 weeks. Weekly wheel running distance was monitored throughout the intervention. Nucleus accumbens (NAc) was assessed for mRNA expression of DA receptors at sacrifice.

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RESULTS—Compared to LCR, HCR ran greater distance and had greater ratio of excitatory/inhibitory DA mRNA expression (both line main effects, $P<0.05$). Wheel running distance was significantly, positively correlated with the ratio of excitatory/inhibitory DA mRNA expression across animals. In both lines, OVX reduced wheel running ($P<0.05$). Unexpectedly, although HCR started with significantly greater voluntary wheel running, they had greater OVX-induced reduction in wheel running than LCR such that no differences were found 11 weeks after OVX between HCR_{OVX} and LCR_{OVX} (interaction, $P<0.05$). This significant reduction in wheel running in HCR was associated with an OVX-mediated reduction in the ratio of excitatory/inhibitory DA mRNA expression.

CONCLUSION—DA system in the NAc region may play a significant role in motivation to run in female rats. Compared to LCR, HCR rats run significantly more, which associates with greater ratio of excitatory/inhibitory DA mRNA expression. However, despite greater inherent motivation to run and an associated brain DA mRNA expression profile, these HCR rats are *not* protected against OVX-induced reduction in wheel running. The impairment in wheel running in HCR rats may be partially explained by their reduced ratio of excitatory/inhibitory DA receptor mRNA expression.

Keywords

ovariectomy; exercise motivation; aerobic capacity; nucleus accumbens; dopamine receptors

1. Introduction

Most Americans fail to meet the U.S. guidelines for physical activity, and postmenopausal women exhibit a particularly high prevalence of physical inactivity for a multitude of reasons [1]. Menopause-associated reduction in physical activity is associated with an increased prevalence of cardiovascular diseases (CVD) such as coronary heart disease and stroke [2, 3]. Conversely, walking and exercise regimens can lower the prevalence of CVD-associated diseases in postmenopausal women [3, 4]. Given the detrimental impact of physical inactivity in postmenopausal women, it is imperative to seek a major psychological/molecular pathway involved in menopause-associated physical inactivity in order to attenuate the CVD-related consequences of menopause, a vicious circle driven by both menopause [5] and consequent physical inactivity [2, 6].

Rats selectively bred for high (HCR) and low (LCR) capacity running also demonstrate a divergence in voluntary wheel running [7], an activity that represents an evolutionarily salient behavior. The large variations in motivated running behavior and locomotion between lines may be mediated by a unique brain pathway to engage motor behavior [8, 9]. The dopamine (DA) system in the mid-brain mesolimbic circuit has been considered a key controller for the regulation of motivation [10], reward [11], and motor control [12], that significantly contributes to voluntary physical activity. Among the DA circuits, the nucleus accumbens (NAc) located in the ventral striatum is a key element in the cognitive and affective processing of voluntary motor actions [13]. DA activation in the NAc plays a critical role in behaviors associated with natural re-enforcers, i.e. voluntary running [14–16]. The role of the DA system in motivating physical activity can be implied from addiction studies [17], in that the DA system serves as a mediator of the incentive or appetitive

properties of rewarding events, such as voluntary running. Roberts and Booth [18] proposed the possibility of an inherent difference in DA-related transcriptomes in the NAc between rats selectively bred for high and low voluntary wheel running, a different type of selective breeding than what was used for the HCR/LCR rats. Despite differences in how selective breeding was performed, the data from Roberts and Booth suggest that higher levels of DA-transcriptomes may also be a mechanism for higher voluntary wheel running in the HCR rats. This argument is supported by previous research demonstrating that highly active mice express greater DA activation in the mesolimbic circuit compared to control mice [7, 19]. While the role of the DA system in the NAc in mediating the rewarding nature of voluntary running has been documented, it is also unknown if elevating excitatory and decreasing inhibitory DA mRNA expression (i.e., HCR rats) protects against OVX-induced reduction in voluntary wheel running and/or locomotion.

Ovarian hormones including estrogen (E_2) appear to exert a tonic stimulation for DA receptors, and chronic stimulation may help maintain DA activation in rodents [13]. Loss of ovarian hormones in OVX rodents, a model of human menopause, reduces wheel running and locomotor activity via attenuated DA receptor expression and DA release at synaptic clefts in the NAc [20, 21]. Recently, our group demonstrated that, while female HCR had ~30% greater spontaneous physical activity (i.e., locomotor activity) than LCR, both HCR and LCR experienced ~20% reduction in physical activity following OVX [22]. As that study employed no wheel running system, we were unable to determine whether OVX in HCR and LCR rats might also alter the re-enforcing nature of physical activity (i.e., voluntary wheel running). The purpose of the present study was to determine 1) if HCR are protected against OVX-associated reduction in voluntary wheel running activity compared to LCR; 2) whether this protection in HCR is associated with preservation of greater excitatory and/or less inhibitory DA mRNA expression in the NAc.

2. Material and methods

2.1 Animal protocol

Using the different levels of performance on 3-grade treadmill exercise tests, we have developed two strains of rats having different intrinsic endurance exercise capacities determined by maximal running distance to exhaustion. We have selectively bred the rats for high (i.e. HCR) and low (i.e. LCR) endurance running capacities over many generations, and finally HCR had ~30% greater intrinsic aerobic capacities compared to LCR rats [23]. Forty female HCR and LCR rats (generation 33) were shipped to the University of Missouri, and singly housed under standard temperature and humidity on a 12h-12h light/dark cycle. Rats were fed with water and standard rodent chow (Harlan Teklad Rodent Diet 8604) *ad libitum*. Animal procedures were approved by Institutional Animal Care and Use Committee (IACUC) at the University of Missouri-Columbia prior to their initiation.

2.2 Experimental design

The HCR and LCR rats were randomized to four groups: HCR_{SHM}, HCR_{OVX}, LCR_{SHM}, and LCR_{OVX}. When the rats were approximately 27 weeks old, sham (SHM) or ovariectomy (OVX) operations were conducted. Following a one-week recovery period, all rats were

given access to voluntary wheel running in their cages, and fed standard rodent chow (Harlan Teklad Rodent Diet 8604) and water *ad libitum* for 11 weeks. Voluntary wheel running distance was monitored weekly. Wheels were locked 3–5 hours prior to sacrifice, to prevent any acute exercise effect. On the day of sacrifice, rats were anesthetized with pentobarbital sodium (100mg/kg; Nembutal Sodium Solution; Akorn, Lake Forest, IL). Immediately after rapid decapitation via guillotine, the nucleus accumbens (NAc) was collected and frozen for further analyses.

2.3 Ovariectomy and sham surgeries

OVX and SHM surgeries were performed as previously described [24]. Briefly, rats were anesthetized with inhaled isoflurane and kept at 2% during surgery. A one-inch incision at midline of the dorsal surface was made, followed by bilateral incisions through the muscle layer to expose the ovaries. After removing the ovaries, the skin incision was closed using wound clips, and rats received acetaminophen (500 mg/kg).

2.4 Brain tissue collection

For mRNA assessment, NAc was collected via a protocol adapted from Mathes et al. [25]. Whole brains were removed and rinsed in saline solution, and then placed posterior side up in an acrylic sectioning apparatus (Braintree Scientific, Braintree, MA). A 2.0 mm coronal slice of each brain was made between Bregma 0.7 and 2.7 mm. Slices were positioned on an ice-cooled plate, and in turn bilateral punches (2.00 mm diameter) were made for the NAc regions. Tissues were stored at –80°C until analysis.

2.5 Voluntary wheel running system

Use of the wheel running system is well established to study rodent voluntary activity [26]. Each cage was equipped with a running wheel (11cm wide with an inner diameter of 35cm). An electronic bicycle computer monitoring system (Sigma Sport BC 800 bicycle computer, Cherry Creek Cyclery, Foster Fall, VA) was connected to the wheel system to quantify the number of revolutions; revolutions were then converted to distance in kilometers.

2.6 Assessment of NAc gene expression

Total mRNA was extracted from NAc tissue from bilateral punches in order to assess gene expression via real-time quantitative PCR (rtPCR). The following mRNAs were measured to assess the DA system: the D1-like DA receptors (excitatory; Drd1 and 5), the D2-like DA receptors (inhibitory; Drd 2, 3, and 4), and dopamine transporter (DAT), and 18S (housekeeping gene). The NAc tissue was thawed on ice, and tissue lysis was conducted by a high-speed shaking apparatus (Tissuelyser LT, Qiagen, Valencia, CA) and Trizol method via manufacturer's instructions. cDNA was reverse transcribed from total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA). Our RNA extraction method was previously validated for optimal quality assurance (i.e., through RNA quality number (RQN) determination); all RQN values were >8.0 [27]. rtPCR was performed using the ABI StepOne Plus sequence detection system (Applied Biosystems) and the iTaq UniverSYBR Green SMX (BioRad, Hercules, CA), as previously described [28]. Primer sequences were designed using the NCBI Primer Design tool and obtained from

IDT (Coralville, IA). 18S was used to amplify the endogenous control product, and mRNA expression values are expressed as 2^{-CT} ($CT = HK\ CT - \text{gene of interest } CT$); gene expression data were expressed relative to the HCR_{SHM} group, which was set to 1.0. The total activation of DA system was estimated by the ratio of the average of D1-like DA receptor gene expressions (i.e. Drd1 and 5) to the average of D2-like DA receptor gene expressions (i.e. Drd 2, 3, and 4).

2.7 Statistical analysis

All data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY). Two-way analysis of variance (ANOVA) was used to determine statistical differences for line main effects (HCR vs. LCR), treatment main effects (SHM vs. OVX), and line $\times$ treatment interactions. For wheel running distance changes over time, two-way ANOVA with repeated measures was used. Bivariate Spearman's correlations were performed to determine the association between the ratio of excitatory to inhibitory DA receptor mRNA expression and total running distance (accumulated running distance for 11 weeks) in HCR and LCR rats. In all cases, $P < 0.05$ was considered statistically different and data are reported as mean $\pm$ SE.

3. Results

3.1 Wheel running assessment

Weekly running distance throughout the 11-week period is depicted in Figure 1A. When averaged over the intervention period, wheel running distance was greater in HCR than LCR and OVX reduced it in both lines compared to SHM group (treatment and line main effect, $F=111$ and $F=83$, respectively, $P < 0.001$ for both main effects; Figure 1B). When investigated as early (i.e., week 1) and late (i.e., week 11) time points, HCR ran greater distance than LCR at both points (line main effect, $F=49.6$ and $F=40.6$ for week1 and week11, respectively, $P < 0.001$ for both time points; Figure 1C), and OVX reduced weekly wheel running distance in both lines at both points compared to SHM group (treatment main effect, $F=9.7$ and $F=56.3$, $P=0.004$ and $P < 0.001$ for week1 and week11, respectively). However, following 11 weeks, HCR had greater OVX-induced reduction in weekly wheel running distance than LCR (time $\times$ treatment; time $\times$ line interactions, $F=3.8$ and $F=2.5$, $P=0.001$ and $P=0.042$, respectively; Figure 1C). Interestingly, although the increase in running over time was significantly lower in LCR_{OVX} compared to LCR_{SHM}, it is important to note that LCR_{OVX} did continue to increase their weekly wheel running from week 1 to week 11. This was in stark contrast to the HCR_{OVX} group, who *decreased* running activity throughout the intervention ($P < 0.001$), an effect not observed in any other group.

3.2 Dopaminergic gene expression in NAc

Overall HCR had greater excitatory dopamine (i.e., Drd1) mRNA expression compared to LCR (line main effect, $F=10.9$, $P=0.003$; Figure 2A). On the other hand, genes associated with inhibitory dopamine (i.e., Drd3 and Drd4) were expressed relatively greater in LCR than HCR rats (line main effect, $F=32.2$ and $F=3.6$, $P < 0.001$ and $P=0.05$ for Drd3 and Drd4, respectively; Figure 2A). OVX differentially affected HCR and LCR rats such that OVX increased inhibitory (i.e., Drd2 and Drd4) mRNA expression in HCR, and reduced those markers in LCR (line $\times$ treatment interaction, $F=18.9$ and $F=11.0$, $P=0.001$ and $P=0.003$ for

Drd2 and Drd4, respectively; Figure 2A). No significant difference was found between line and treatment in Drd5 and DAT mRNA expression. The estimated marker of DA activation, assessed by the ratio of excitatory DA receptor to inhibitory DA receptor gene expressions, was greater in HCR than LCR (line main effect, $F=15.7$, $P<0.001$), but OVX only affected this ratio in HCR (line $\times$ treatment interaction, $F=4.7$, $P=0.037$; Figure 2B). Moreover, this ratio was positively correlated with weekly running distance across animals ($r=0.55$, $F=167$, $P<0.001$, Figure 2C). This significant correlation across all animals was primarily attributed to the significant correlation in HCR rats ($r=0.43$, $F=52$, $P<0.05$; Figure 2D) whereas the correlation was not significant in LCR rats alone ($r=-0.004$, $F=-0.245$, $P=0.49$; Figure 2E). However, the correlation was strengthened with the addition of the LCR rats, suggesting that the relationship exists in both lines.

4. Discussion

It is well established that loss of ovarian hormones (e.g., rodent ovariectomy (OVX) and human menopause) reduces spontaneous physical activity [22, 29, 30]; yet, whether female hormone loss affects motivated physical activity (e.g. wheel running in rodents or structured exercise in humans) is not known. It is also known that physiological and psychological drives for physical activity are mediated via brain dopamine (DA) system [14–16]; which may cause the HCR rats to be physically active [18]. Yet, if elevating DA receptor expression (i.e., HCR rats) protects against OVX-induced reduction in voluntary wheel running is uncertain. The major findings of the present study were as follows. First, similar to our previous findings that OVX reduced spontaneous physical activity in both HCR and LCR rats [22], both lines experienced an OVX-mediated reduction in voluntary wheel running (i.e., motivated running behavior) compared to their SHM counterparts. Interestingly, HCR_{OVX} was the only group that did not escalate weekly wheel running from week 1 to week 11, while LCR_{OVX} showed attenuated increase of wheel running over time compared to LCR_{SHM}. Second, HCR rats also appeared susceptible to an OVX-mediated reduction in the ratio of excitatory/inhibitory DA receptor mRNA expression while the ratio was unchanged in LCR rats following OVX. Strikingly, this change in DA receptor mRNA expression in HCR rats was associated with a significant reduction in wheel running from week 1 to week 11. Finally, compared to LCR rats, HCR did have a significantly greater ratio of excitatory/inhibitory DA receptor mRNA expression compared to LCR rats; and that ratio correlated significantly in the positive direction with voluntary wheel running across animals. That relationship was driven largely by the HCR line, as the correlation was not significant in LCR rats alone (Figure 2C–E).

Approximately 60% of all women fail to meet the U.S. guidelines of physical activity [1]. Moreover, women over age 60 are a particularly concerning cohort regarding physical inactivity; indeed, a strong, inverse association between physical activity level and mortality has been reported in postmenopausal women [1]. The reason why this group is particularly susceptible to physical inactivity is not well understood, but there is a strong association between loss of ovarian hormones and reduced physical activity [30]. Using OVX rodent models, previous research has demonstrated that lack of ovarian hormones *per se* causes a reduction in spontaneous physical activity (i.e. locomotor activity) [21], and decreases voluntary running activity [20, 31, 32]. We previously showed that both HCR and LCR rats

experienced a ~20% reduction in spontaneous physical activity following OVX, but HCR_{OVX} still had enhanced SPA levels compared to LCR_{OVX} [22]. Rodent wheel running is an accepted model of voluntary, motivated activity, and has also been used to study the brain reward mechanism associated with voluntary activity [26]. The present study showed that OVX reduced voluntary wheel running in both HCR and LCR rats. In week 1, HCR appeared to be somewhat protected from an OVX-induced reduction in wheel running, such that OVX reduced wheel running distance more in LCR (HCR_{OVX} : -45% compared to HCR_{SHM} ; LCR_{OVX} : -65% compared to LCR_{SHM} ; Figure 1C), and HCR_{OVX} still ran more than 10-fold greater in wheel running distance compared to LCR_{OVX} . However, only the HCR_{OVX} group gradually decreased their wheel running over time so that, by week 11, HCR_{OVX} and LCR_{OVX} groups ran a similar weekly running distance (Figure 1A and C), and OVX-induced reduction in wheel running distance was greater in HCR than LCR (HCR : -75% compared to HCR_{SHM} ; LCR : -65% compared to LCR_{SHM} ; a significant interaction, $P < 0.005$; Figure 1C). These data suggest that HCR are more susceptible to OVX-associated attenuation in wheel running than LCR on a longer-term basis (i.e., following 11 weeks). This may be explained by the fact that HCR started with greater wheel running distance than LCR, thus they had more distance to decline. On the other hand, LCR started at an already very low activity level with almost no room to decline.

Knab and Lightfoot [16] stated that DA system in the NAc is a major controller of voluntary running. Other research has implicated the mesolimbic DA circuits in the regulation of motivation [10], reward [11], and motor control [12], all of which may contribute to voluntary activity. Among the DA circuits, NAc located in the ventral striatum is a key element in the cognitive and affective processing of voluntary motor actions [13]. The DA neurons in the striatum appears to be responsible for control of motor activity [33], while DA activity in the NAc plays a critical role in behaviors associated with natural re-enforcers, e.g. sexual behavior, food consumption, incentive learning, or voluntary running [14, 15, 34]. The role of the DA system in motivating physical activity can be implied from addiction studies [17, 35], in that the DA system may mediate the incentive and reward properties that motivate an animal to perform additional physical activity. A most salient finding of the present study is that wheel running distance was positively correlated with the ratio of excitatory/inhibitory DA receptor mRNA expression, measured by the ratio of excitatory to inhibitory receptors across animals, suggestive of the significant role of DA system in wheel running. No studies have previously showed the importance of DA system in regulating motivated wheel running activity using the ratio of excitatory to inhibitory receptor gene expression as a marker of estimated DA activation. Our finding is partially congruent with a previous animal study [36] demonstrating that DA knock-out rodents have reduced motivation for high-effort tasks. Moreover, using a DA receptor-specific agonist, De Vries et al. [37] demonstrated the importance of the DA system in regulating physical activity by injecting a *Drd1* (i.e., excitatory) receptor agonist into the NAc and showing a resultant enhancement in physical activity. Baldo et al. [38] further confirmed this finding, showing that injecting a *Drd1* receptor antagonist reduces physical activity. Thus, it is evident that brain DA system dictates physical activity behavior in rodents. For the first time, the present study demonstrates that excitatory DA receptor gene expression (i.e., *Drd1*) gene expression was greater in HCR (well characterized as having intrinsically high levels of both

spontaneous and wheel running activities) compared to LCR rats (characterized as having the counter phenotype – lower spontaneous and wheel running activities). Similarly, we found that inhibitory DA receptor gene expression (i.e., *Drd3* and *Drd4*) were lower in HCR.

We tested the hypothesis that HCR rats would be protected against OVX-induced reduction in wheel running compared to LCR due to greater baseline DA activation (i.e. increased excitatory and decreased inhibitory DA receptors) in the NAc. We did show that HCR_{SHM} exemplified significantly greater voluntary wheel running than LCR_{SHM} rats, and that this enhancement was paralleled by a similar enhancement in the ratio of excitatory/inhibitory DA receptor mRNA expression. Yet, an unexpected finding was that HCR_{OVX} was the only group that did not increase weekly wheel running from week 1 to week 11. It has been shown that HCR have more than 5-fold greater voluntary running distance compared to LCR rats [9], and Booth's group [18] previously proposed the possibility that rats selectively bred for high voluntary running are more physically active owing to their enhanced DA-associated transcriptomes in the NAc. Previous studies with mice also demonstrated that highly active mice express greater DA activation in the mesolimbic circuit compared to control mice [7, 19]. However, we found that, despite greater ratio of excitatory/inhibitory DA receptor mRNA expression, HCR were not protected against OVX-mediated reduction in wheel running. Specifically, HCR_{OVX} *decreased* wheel running from week 1 to week 11 while LCR_{OVX} *increased* running over time. This differential response to OVX was paralleled by a unique interaction response in two of the DA inhibitory receptors, notably, *Drd 2* (Figure 2A) such that OVX increased gene expression in HCR and decreased it in LCR compared to their SHM counterparts. The reason for this interesting interaction is not clear. However, it has been demonstrated in rats that OVX significantly increases *Drd 2* receptor density and DA binding in the NAc brain region, which is normalized with estrogen replacement [39]; indeed other reports show that estrogen decreases *Drd 2* receptor density in the NAc [40]. Thus, the effect we observed in the LCR rat following OVX appears to be the atypical response. Similar to that study by Chavez et al. [39], we did not observe any changes in *Drd 1* expression following OVX; thus, our data support their hypothesis that changes in DA receptor levels following OVX are specific to *Drd 2*. However, it is important to re-acknowledge here that our data were mRNA only, whereas Chavez et al. measured receptor levels and binding affinities. Thus, more studies are clearly necessary to investigate the mechanism and implications of this differential response to OVX in *Drd 2* expression between HCR and LCR rats. As an exploratory analysis, we expressed the mRNA levels of excitatory and inhibitory DA receptors as a ratio and compared the groups. Although the meaningfulness of this measure is not completely clear given that it only accounts for differences in relative gene expression, it is interesting to note that only HCR rats experienced a ~30% reduction in the excitatory/inhibitory ratio following OVX, which was significantly associated with their reduction in wheel running over time. Thus, although not conventional, we felt compelled to present the data that way given the significant correlation with running behavior.

It is unclear why OVX reduces wheel running in HCR rats despite their intrinsically enhanced DA receptor mRNA expression, but it may be partially attributed to a strong effect of ovarian hormones on mediating DA activation and physical activity, independent of intrinsic aerobic capacity level. We also recently demonstrated a strong impact of ovarian

hormones on metabolic function (i.e., tissue-specific insulin sensitivity) independent of intrinsic aerobic capacity level [41]. Among ovarian hormones, circulating E₂ is the critical mediator of physical activity in female animals. One study suggests estrogenic modulation of neurotransmitters including DA in mid-brain [42]. E₂ appears to exert a tonic stimulation for striatal DA receptors, and chronic stimulations may help maintain DA activity in rodents. Whereas this stimulation is lost following OVX, E₂ replacement immediately after OVX can preserve the upregulated D1-like (excitatory) DA receptors [43, 44]. The estrogenic pathway can also control DA functioning in different steps such as DA release and metabolism at the pre- and post- synaptic receptors [45]. Nearly all areas of the brain present DA receptor expression, while greater expression of the receptors were found in mesolimbic/nigrostriatal circuits [46]. In summary, ovarian hormones including E₂ likely play a critical role in mediating motivation to physical activity via activation of DA system. Loss of E₂ by OVX may decrease motivated physical activity through an attenuation of DA activation independent of intrinsic physical activity level, but likely involves other factors related to ovarian hormone loss. Future studies are needed to investigate the underlying mechanisms by which HCR_{OVX} was the only group that did not increase weekly wheel running from week 1 to week 11, and also showed reduced ratio of excitatory/inhibitory DA receptor mRNA expression compared to HCR_{SHM}.

The findings of the present study should be considered along with its limitations. First, we used the gene expression ratio of excitatory to inhibitory DA receptors as an estimate of DA activation. This ratio may not represent the actual DA activation, and some studies reported the importance of the binding state or affinity of receptors to DA rather than the quantitative presence of DA receptors [47, 48]. Nonetheless, studies still argue the importance of altered gene transcription for specific DA receptors, to investigate reward and motivation for physical activity [18, 49]. Future studies should employ more substantive methods (e.g., immunohistology, protein analyses, binding affinity assays) to validate the gene expression data described herein. Yet, the fact that our gene expression analyses correlate so strongly with the physiological/behavioral data may argue for the validity of these measures. The second limitation is that the HCR/LCR model provides two extreme conditions with contrasting running capacity, and it may be argued that a true control is lacking. However, we felt that this model served as a good means to initially address the question of whether aerobic fitness affects voluntary running behavior following loss of ovarian hormones. Future studies will assess how exercise training affects this response to OVX in terms of running behavior and brain changes. Similarly, we did not have true sedentary controls (e.g. a wheel-locked control). Because our question was about the role of fitness in protecting against OVX-related reductions in voluntary behavior, we providing all groups with running wheels to control for differences in running behavior over time under ovary-intact conditions. Future studies should measure brain changes following OVX versus SHM in HCR versus LCR rats also under sedentary conditions. Certainly, more mechanistic studies are required to determine causal relationships between OVX-mediated changes in brain dopamine signaling and/or behavior.

4. Conclusions

Despite having a greater ratio of excitatory/inhibitory DA receptor mRNA expression in the NAc brain region compared to low-fit LCR rats, high-fit HCR rats were not protected against an OVX-induced reduction in voluntary wheel running. Remarkably, this reduction in wheel running following OVX, observed especially in HCR rats, correlated significantly with a reduction in the ratio of excitatory/inhibitory DA receptor mRNA expression in the NAc. Indeed, average wheel running distance throughout the intervention correlated positively and significantly with that excitatory/inhibitory ratio across all animals. In conclusion, NAc brain region DA receptor gene expression is affected by OVX and may result in reduced motivated physical activity in female rats. Thus, activation of DA receptors may serve as a future therapeutic target to enhance motivation for physical activity in postmenopausal women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- High capacity running (HCR) rats have greater net positive dopamine (DA) receptor gene expression in the nucleus accumbens (NAc) brain region compared to low capacity running (LCR) rats.
- NAc DA receptor gene expression significantly correlates with voluntary wheel running.
- Ovariectomy (OVX) reduces voluntary wheel running independent of intrinsic running capacity.
- Compared to LCR, HCR rats experience *greater* OVX-induced reduction in wheel running which associates with a *greater* reduction in net positive DA receptor gene expression.

Figure 1. Wheel running behavior

(A) weekly wheel running distance curves for 11 weeks, (B) averaged running distance, and (C) wheel running distance in Week 1 and 11. Values are means $\pm$ SE ($n=8-10$ per group). Line = line main effect, HCR vs. LCR; Treatment = treatment main effect, SHM vs. OVX; Interaction = line $\times$ treatment interaction. * Denotes that HCR had greater reduction following OVX than LCR.

Figure 2. Nucleus accumbens dopamine receptors

(A) dopamine mRNA expressions (excitatory: dopamine receptor (Drd)1 and 5; inhibitory: Drd 2, 3, and 4; dopamine transporter (DAT)); (B) estimated DA activation (ratio of excitatory to inhibitory receptors); (C) association of the ratio of excitatory/inhibitory DA receptor mRNA expression and total running distance (accumulated running distance for 11 weeks); (D) association of the DA receptor ratio and total running distance in HCR; and (E) association of the DA receptor ratio and total running distance in LCR. Data are expressed relative to HCR_{SHM}. Values are means $\pm$ SE ($n=8-10$ per group). Line = line main effect, HCR vs. LCR; Treatment = treatment main effect, SHM vs. OVX; Interaction = line $\times$ treatment interaction. ‡ Denotes difference ($P < 0.05$) between SHM and OVX within line. * $P < 0.001$, significant correlation between the ratio of excitatory/inhibitory DA receptor mRNA expression and running distance.

Table 1

Body composition and metabolic markers

	HCR		LCR		2-way ANOVA statistics	
	SHM	OVX	SHM	OVX		
Body weight (g)	248.09 ± 8.61	283.84 ± 8.78	277.52 ± 5.10	292.38 ± 7.39	Line, F=6.6, P=0.014 Treatment, F=11.8, P=0.002	
Lean mass (g)	235.20 ± 8.70	255.24 ± 6.61	256.78 ± 1.65	269.82 ± 6.86	Line, F=7.9, P=0.008 Treatment, F=6.6, P=0.015	
Fat mass (g)	12.89 ± 0.83	28.59 ± 4.75	20.76 ± 3.75	22.57 ± 2.64	Treatment, F=8.3, P=0.007 Line × Treatment, F=5.2, P=0.028	
Fat percent (%)	5.25 ± 0.39	9.90 ± 1.42	7.35 ± 1.14	7.69 ± 0.82	Treatment, F=7.2, P=0.011 Line × Treatment, F=5.4, P=0.027	
BMD (g/cm ²)	0.171 ± 0.002	0.164 ± 0.002	0.173 ± 0.002	0.169 ± 0.002	Treatment, F=4.8, P=0.035	
Uterus (g)	0.904 ± 0.07	0.196 ± 0.02	0.828 ± 0.06	0.201 ± 0.02	Treatment, F=189, P<0.001	
GTT Glucose (tAUC; mg/dl*min)	20827 ± 2621	21305 ± 2136	22772 ± 2014	21779 ± 2368	NS	

Two-way ANOVA for line (i.e., HCR vs. LCR), treatment (i.e., SHM vs. OVX) and line × treatment interaction effects were determined; values are means ± SE (n=7–10 per group). BMD= bone mineral density; GTT = glucose tolerance test assessed by glucose total area under curves (tAUC) during GTTs.