Brain Research 1698 (2018) 187–194

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Medial habenula maturational deficits associate with low motivation for voluntary physical activity

Kolter B. Grigsby[a,*], Taylor J. Kelty[a], Frank W. Booth[a,b,c,d]

[a] Department of Biomedical Sciences, University of Missouri, Columbia, MO, United States
[b] Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, MO, United States
[c] Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO, United States
[d] Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO, United States

HIGHLIGHTS

- HVR rats show higher expression of MHb developmental genes than LVR rats.
- *Brn3a* and *Nurr1* mRNA expression are positively correlates with HVR run distance.
- HVR rats have increased expression of neuronal maturation markers compared to LVR.
- LVR rats show lower dendritic density and higher thin spine percentage than HVR.

ARTICLE INFO

Keywords:
Voluntary wheel-running behavior
Medial habenula
Neuronal maturation
Motivation

ABSTRACT

The habenula is a small, diencephalic structure comprised of distinct subnuclei which receives inputs from the limbic forebrain and sends projections to various regions in the midbrain, making this region well positioned to influence reward and motivation. Genetic ablation of the dorsal medial habenula is known to decrease voluntary wheel-running in mice. However, the extent to which the medial habenula (MHb) mediates wheel-running motivation in the context of high or low motivation for voluntary physical activity remains to be determined. In so, we utilized 5-week-old female rats selectively bred to voluntarily run high (HVR) or low (LVR) distances in order to determine if inherent differences in medial habenula maturation accompany inherent differences in wheel-running motivation. We report a significantly higher expression of genes associated with MHb development (*Brn3a*, *Nurr1*, *Tac1,* and *Kcnip*) in HVR versus LVR rats. Furthermore, there was a positive correlation between *Brn3a* and *Nurr1* expression and run distance in HVR, but not LVR rats. Similarly, NeuN and Synapsin 1, markers of neuronal maturation, were higher in HVR compared to LVR rats. Lastly, dendritic density was determined to be higher in the MHb of HVR versus LVR rats, while LVR rats showed a higher percentage of thin spines, suggesting a higher prevalence of immature dendrites in LVR rats. Taken together, the above findings highlight the involvement of MHb in driving the motivation to be physically active. Given pandemic levels of global physical inactivity, the role of the MHb offers a novel potential to improve our global health.

1. Introduction

In light of staggering health care costs in the U.S., physical inactivity stands as a major, albeit largely ignored, contributor to declining human health (Troiano et al., 2008). Recent evidence suggests that physical inactivity is associated with 40 known chronic diseases and conditions (Ruegsegger and Booth, 2017), a concerning reality given that approximately 97% of U.S. adults do not meet reach U.S. recommendations for daily physical activity (Troiano et al., 2008). More globally, the World Health Organization has categorized physical inactivity as the 4th leading risk factor for worldwide death, which contributed to ~6% of global deaths in 2008 (Who, 2010). In so, a better understanding of neuro-processes that underlie the motivation to be physically active has vast potential to greatly influence our global health.

Despite evident environmental factors influencing human daily activity, a study which employed 772 mono- and dizygotic twin pairs determined that ~30% of the contribution to human sedentary

* Corresponding author at: Department of Biomedical Sciences, University of Missouri-Columbia, E102 Veterinary Medicine Bldg., 1600 E. Rollins, Columbia, MO 65211, United States.
E-mail address: kgtg2@mail.missouri.edu (K.B. Grigsby).

https://doi.org/10.1016/j.brainres.2018.08.016
Received 27 April 2018; Received in revised form 11 August 2018; Accepted 13 August 2018
Available online 14 August 2018
0006-8993/

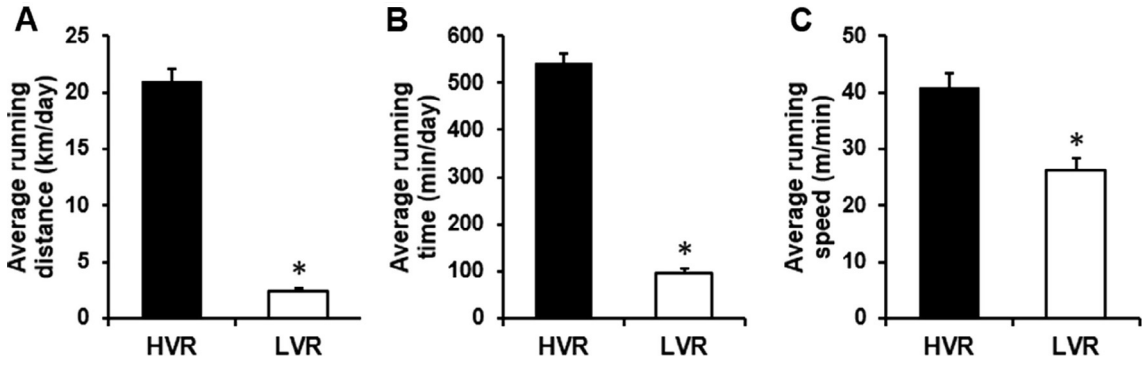

Fig. 1. Average daily running distance, time and velocity (± SEM) for HVR (n = 6) and LVR (n = 8) over 5-day running period between 28 and 35 days of age. (A) Average run distance (km/day) for HVR and LVR rats. (B) Average run time (min/day) for HVR and LVR rats. (C) Average running velocity (m/min) for HVR and LVR rats. *denotes significantly different ($p < 0.05$) compared to HVR.

behavior is genetic (den Hoed et al., 2013). Preceding the notion of genetic determinants of human physical inactivity, the Booth lab had published a selectively bred rat model of high (HVR) and low (LVR) voluntary wheel-running behavior (Roberts et al., 2013, 2012). Given the positive motivational quality of voluntary wheel-running in rodents, we contend that the selectively bred HVR and LVR polygenic models capture the likely existence of gene pools for “high” and “low” physical activity motivation.

The mesolimbic dopamine system has garnered much attention for its role in reward and motivation (Baik, 2013). Although neuromolecular mechanisms responsible for voluntary physical activity motivation remain elusive, special attention has been placed on the dopaminergic striatum, and in particular the nucleus accumbens (NAc), in driving these behaviors (Knab et al., 2009; Knab and Lightfoot, 2010). Equally as important to the establishment and mediation of motivational and rewarding information is the role of limbic nuclei, namely the amygdala, hippocampus and prefrontal cortex circuits (Vanderschuren and Kalivas, 2000). At the interface of both circuits lies the habenula, a small, diencephalic structure that sends projections to the striatum and receives input from areas of the limbic system (Viswanath et al., 2014).

Substantial interest has been placed on elucidating the role of lateral habenula in driving negative motivational signaling (Matsumoto and Hikosaka, 2007). Evidence suggests that the lateral habenula (LHb) regulates negative motivation, or rather aversion, through inhibitory interactions with the dopaminergic rostromedial tegmental nucleus (Jhou et al., 2009), as well as through regulation of striatal serotonin release (Kalén et al., 1989). Despite the importance of this region in mediating aversive signaling, evidence suggests that the adjacent, and notably distinct medial habenula (MHb) is potentially important in regulating stress (Lecourtier et al., 2004) and depression (Shumake et al., 2003). Furthermore, the interconnected posterior septum and the medial habenula, the septo-habenular pathway, is thought to be a crucial intermediary between the limbic system and the midbrain (Lecourtier and Kelly, 2007; Sutherland, 1982). Yamaguchi *et al.* nicely demonstrated that the subdivisions of the posterior septum, the triangular septum (TS) and the bed nucleus of the anterior commissure (BAC) constitute two parallel pathways that project to the ventral (vMHb) and medial (mMHb) aspects of the MHb, respectively (Yamaguchi et al., 2013). By use of immuno-toxin mediated ablation, Yamaguchi *et al.* also demonstrated the role of the TS-vMHb projection in controlling anxiety-related behavior, as well as the role of the BAC-dMHb projection in regulating fear-related behaviors (Yamaguchi et al., 2013). However, Hsu *et al.* demonstrated that genetic ablation of the dMHb using tissue specific deletion of *Pouf1* (*Brn3a*) had no effect on contextual conditioned fear response compared to controls, suggesting the dMHb may have little significant role in the acquisition of fear response or contextual conditioned fear (Hsu et al., 2016). Further, unidirectional connections between the MHb and LHb have been shown, possibly suggesting a potential control process over LHb activity and thus indirectly influencing positive motivation (Kim and Chang, 2005), though no direct evidence of this connection has been elucidated. In so, more attention needs to be placed on uncovering the role of this region in reward and motivation, and in particular the motivation to be physically active.

Foundational work has shown that the medial habenula is genetically distinct from the lateral habenula (Wagner et al., 2016). Quina *et al.* determined that *Brn3a* and *Nurr1* form a regulatory pathway that is necessary for medial habenula development (Quina et al., 2009). Utilizing this known molecular machinery, selective deletion of *Pouf1* (*Brn3a*) in the dorsal medial habenula was shown to decrease voluntary wheel-running behavior in mice (Hsu et al., 2014). Building off this seminal work, we sought to determine if deficits in *Brn3a* and *Nurr1* expression, alongside maturational insufficiencies in the medial habenula, underlies the low voluntary wheel-running behavior seen in our LVR rats. In so, our goals were to determine 1.) If low levels of expression of the *Brn3a* regulatory pathway are associated with selective breeding for low voluntary wheel-running behavior, 2.) If expression of neuronal maturation markers in the medial habenula are endogenously higher in HVR compared to LVR rats and 3.) If dendritic density and morphology, both markers of neuronal maturity and synaptic function, in the MHb accompany differences in voluntary wheel-running phenotype. We hypothesized that *Brn3a* and associated genes would be inherently lower in LVR compared to HVR rats. Further, we additionally hypothesized that this would associate with decreased maturation of MHb neurons in LVR rats. Such a reduction in MHb maturity could, in part, contribute to the characteristically low motivation to be physically active in our LVR rats.

2. Results

2.1. Five-week old HVR and LVR voluntary wheel-running behavior phenotypes

Average daily running distance, time, and speed for 5-wk-old HVR (n = 6) and LVR (n = 8) rats that had voluntarily run between 28 and 34 days of age are shown in Fig. 1A, B, and C, respectively. There was a noticeable ~10-fold higher run distance in HVR than LVR rats, as well as a ~6-fold higher average running time (Fig. 1A and B). Similarly, there was a significant difference in average running speed between HVR and LVR rats (40.74 ± 2.64 m/min vs. 26.08 ± 2.25 m/min; $p = 0.03$; Fig. 1C), suggesting that beyond running further and for a longer period of time, HVR rats also ran at a higher intensity, on average, than LVR rats. These findings remain consistent with historical records of differences in running behavior between our HVR and LVR rats (Roberts et al., 2013; Ruegsegger et al., 2015).