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Striatal Enkephalinergic Differences in Rats Selectively Bred for Intrinsic Running Capacity

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Abstract

Rats selectively bred for high- and low-capacity for running on a treadmill (HCR; LCR) also differ in wheel-running behavior, but whether wheel-running can be explained by intrinsic or adaptive brain mechanisms is not as yet understood. It is established that motivation of locomotory behavior is driven by dopaminergic transmission in mesolimbic and mesostriatal systems. However, whether voluntary wheel running is associated with enkephalinergic activity in the ventral striatum is not known.

Materials & Methods—40 male (20 HCR and 20 LCR) and 40 female (20 HCR and 20 LCR) rats were randomly assigned to 3 weeks of activity wheel exposure or sedentary conditions without wheel access. After 3 weeks of activity-wheel running, rats were decapitated and brains were extracted. Coronal sections were analyzed utilizing *in situ* hybridization histochemistry for enkephalin (ENK) mRNA in the ventral striatum.

Results—HCR rats expressed less ENK than LCR rats in the nucleus accumbens among females ($p < .01$) and in the olfactory tubercle among both females ($p < .05$) and males ($p < .05$). There was no effect of wheel running on ENK mRNA expression.

Conclusion—Line differences in ENK expression in the olfactory tubercle, and possibly the nucleus accumbens, partly explain divergent wheel-running behavior. The lower striatal ENK in the HCR line is consistent with enhanced dopaminergic tone, which may explain the increased motivation for wheel running observed in the HCR line.

Keywords

Enkephalin; Nucleus Accumbens; Olfactory tubercle; Activity wheel; *in situ* hybridization

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1. Introduction

Family and twin studies indicate that variation in human physical activity levels is heritable (Eriksson et al., 2006; Simonen et al., 2002; Stubbe et al., 2006), but the genetic determinants of physical activity are poorly understood (Dishman, 2008). Voluntary wheel running by rodents also has a genetic component (Knab and Lightfoot, 2010; Lightfoot et al., 2004; Lightfoot et al., 2008; Roberts et al., 2013; Swallow et al., 1998; Waters et al., 2013). Rats selectively bred at the University of Michigan for high-capacity running (HCR) or low-capacity running (LCR) (Koch and Britton, 2001) demonstrate substantial divergence in treadmill performance, including running speed and distance (Høydal et al., 2007; Koch and Britton, 2008) and also daily wheel-running (Groves-Chapman et al., 2011; Waters et al., 2008), an activity that appears to represent a preferred and evolutionarily salient behavior in rodents (Belke and Wagner 2005; Brené et al., 2007; Iversen, 1993; Lett, 2000; Sherwin, 1998).

The HCR line is associated with several traits subordinate to exercise performance, including a greater capacity to deliver and utilize O₂ in skeletal muscle (Howlett et al., 2009; Gonzalez et al., 2006), but these differences do not fully account for the large differences in running behavior between lines. Instead, these variations may reflect traits that mediate the relationship between a central drive to engage in motor behavior and observed locomotion (Jónás et al., 2010; Novak et al., 2010). The HCR and LCR rats provide a model from which the brain pathways underlying heritable running behavior and gene-environment interaction can be investigated (Koch and Britton, 2008).

Although the neurobiology of motivated wheel running is as yet unknown, there is substantial evidence for a mechanism involving the mesolimbic-motor interface (Burgess, 2010; Knab et al., 2009; Scheurink et al., 2010). The cumulative evidence suggests this junction exists at the basal ganglia (Garcia-Rill, 1986; Mogenson, 1987; Parent and Hazrati, 1995; Smith et al., 1988; Takakusaki et al., 2004), particularly in striatal GABA/opioidergic neurons located in distinct areas of the striatum that receive dopaminergic projections from the ventral tegmental area (Cardinal et al., 2002; Depue and Collins, 1999; Horvitz, 2002).

Striatal GABAergic medium spiny neurons express D2-like dopamine receptors and enkephalin or D1-like receptors and dynorphin in the direct (striatonigral) pathway and indirect (striatopallidal) pathway, respectively (Gerfen and Young, 1988; Surmeier et al., 1996). Midbrain dopaminergic transmission sensitizes the striatum to rewarding stimuli, mediates the incentive salience associated with these stimuli (Berridge and Robinson, 1998; Ikemoto, 2007; Morales-Mulia, 2013), increases in response to acute (Hattori et al., 1994) and chronic treadmill training (Gilliam et al., 1984), and is up-regulated in the striatum of mice selectively bred for high levels of activity-wheel running (Mathes et al., 2010). The motivational drive to run is plausibly mediated by striatal enkephalinergic neurotransmission in the nucleus accumbens septi (NAS) and olfactory tubercle (OT) or through the efferent targets of these neurons in the ventral pallidum (LeMoine et al., 1990; Young et al., 1986). The striatal enkephalin-dopamine environment may be important for understanding voluntary locomotory behavior (Dishman & Holmes, 2012; Kalivas et al., 1983). Enkephalin (ENK) is a peptide neuromodulator of GABAergic projections to the ventral pallidum (the

limbic structure contiguous with motor pathways) that appears to suppress motor activity and motivated behavior (Durieux et al., 2009; Ena et al., 2011; Kravitz et al., 2013). Wheel-running behavior in rats may be directly attributable to differences in ENK expression (Werme et al., 2002), and divergent running performance observed between HCR and LCR rats may be explained by differences in striatal ENK expression. We hypothesized that HCR rats would have less ventral striatal ENK expression than LCR rats and that three weeks of access to wheel-running would down-regulate ENK expression in the ventral striatum compared to a sedentary housing condition.

2. Results

2.1 Running Distance and Body Weight

Weekly running was reliable across the three weeks in females, intraclass correlation (ICC) (2,3) = .875 and in males, ICC (2,3) = .900. Running increased over time in females, $F(2,36)=14.486$, $\epsilon=.846$, $\eta^2 = .45$, $p<.001$, and males, $F(2,36)=4.45$, $\epsilon=.980$, $\eta^2 = .20$, $p<.05$. There was an effect of line in females, $F(1, 18)=47.289$, $\eta^2 = .72$, $p<.001$, and in males, $F(1, 18)=13.766$, $\eta^2 = .43$, $p<.01$. HCR rats ran more on average than LCR rats, but there was also a line $\times$ quadratic trend across time in females, $F(1,18) = 10.192$, $\eta^2 = .36$, $p=.005$. There was a quadratic effect of time independent of line in males, $F(1,18) = 4.927$, $\eta^2 = .22$, $p=.04$. Among females, weekly running distance increased linearly in LCR, $F(1,9) = 12.212$, $\epsilon=.564$, $\eta^2 = .58$, $p=.007$, but it reached a plateau after week 2 in HCR, $F(1,9) = 8.168$, $\epsilon=.908$, $\eta^2 = .48$, $p=.017$. Among males, running distance increased linearly in LCR, $F(1,9) = 8.805$, $\epsilon=.908$, $\eta^2 = .50$, $p=.016$ (Fig. 1).

Body weight was reliable across the five weeks in females, ICC (2,3) = .978, and in males, ICC (2,3) = .986. Body weight increased linearly over time in females, $F(2,72)=653.246$, $\epsilon=1.0$, $\eta^2 = .95$, $p<.001$, and in males, $F(2,72)=954.299$, $\epsilon=1.0$, $\eta^2 = .964$, $p<.001$. There was an effect of line in females, $F(1, 36)=126.081$, $\eta^2 = .78$, $p<.001$, and in males, $F(1, 36)=110.919$, $\eta^2 = .76$, $p<.001$. HCR rats weighed less on average (initial mean $\pm$ SD, pre-decapitation mean $\pm$ SD) (females: 163 ± 11 g, 204 ± 17 g; males: 230 ± 18 g, 318 ± 35 g) than LCR rats (females: 209 ± 15 g, 259 ± 16 g; males: 330 ± 38 g, 438 ± 48 g), but line and line $\times$ condition effects were not significant between wheel running rats (females: 184 ± 27 g, 223 ± 33 g; males: 277 ± 57 g, 366 ± 66 g), and SED rats (females: 187 ± 28 g, 239 ± 31 g; males: 283 ± 61 g, 391 ± 81 g) (females: F -values < 3.2 , $\eta^2 < .08$, p -values $> .08$; males: F -values < 2.4 , $\eta^2 < .07$, p -values $> .13$).

2.2 ENK mRNA in the Striatum

There was a line effect in the NAS among females, $F(1,25)=13.038$, $\eta^2 = .343$, $p=.001$, and the OT among males, $F(1,30)=7.510$, $\eta^2 = .20$, $p=.01$ and females, $F(1,25)=4.924$, $\eta^2 = .165$, $p=.036$. HCR expressed less ENK mRNA compared to LCR in the ventral striatum (Fig. 2a). A similar effect in the dorsal striatum did not reach statistical significance for females, $\eta^2 = .13$, $p=.067$, or males, $\eta^2 = .03$, $p=.29$ (Fig. 2b). There were no condition, condition $\times$ line, or condition $\times$ line $\times$ sex effects of wheel running on ENK mRNA (F -values < 1.815 , $\eta^2 < .032$, $p > .183$).

Among females, average running distance was correlated with ENK mRNA in the NAS, $r = -.740$, $t = 3.48$, $p = .006$, and the OT, $r = -.631$, $t = 2.57$, $p = .028$ (Fig 3). Among males, ENK mRNA was uncorrelated with running distance in the OT ($r = .069$, $t = .26$, $p = .80$). Dorsal striatal ENK mRNA was uncorrelated with running distance in females, $r = -.479$, $t = 1.724$, $p = .115$, or males, $r = .109$, $t = .453$, $p = .656$.

3. Discussion

We report that HCR rats expressed less ENK than LCR rats in the NAS among females and in the OT among both females and males. Sex differences seen here in striatal ENK expression among rats selectively bred for divergent treadmill performance are consistent with those described in non-selectively bred rats (Tang and Man, 1991). High intrinsic drive to run, evidenced here by four- to six-fold greater distances run each day by HCR rats, was marked by low ENK mRNA expression in the ventral striatum, suggesting that a central opioidergic mechanism underlies voluntary running behavior. Multiple lines of evidence conclude that ENK projections from the ventral striatum directly modulate neural activity in the ventral pallidum to inhibit motivated locomotory behavior (Haber, 2011) and that low ENK gene expression may reflect increased dopaminergic tone (Carta et al., 2001; Gerfen et al., 1991; Li et al., 1990; Nikoshkov et al., 2008). Reduced ENK may elevate striatal opioidergic receptor expression, sensitizing the organism to natural reward and promoting wheel-running behavior in HCR rats (Greenwood et al., 2011). Improved animal models and epigenetic human studies are necessary to further elucidate the effects of motivational and hedonic processing on exercise (Dishman and Holmes, 2012).

Three weeks of exposure to wheel running had no effect on ventral or dorsal striatal ENK transcription compared to sedentary housing, consistent with a prior report on female rats (Bjornebekk et al., 2005). It has been demonstrated that thirty days of wheel access reduces

FosB expression in enkephalinergic cells of the NAS (Werme et al., 2002) and that eight weeks of treadmill training blunts ENK release in the basal ganglia after a fatiguing bout of treadmill running (Blake et al., 1984). Although nine days of wheel access increased hippocampal ENK in spontaneously hypertensive rats (Persson et al., 2004), it appears that three weeks of wheel running is not sufficient to induce ENK changes in the striatopallidal pathway. Here, intrinsic transcription of ENK was negatively correlated with average running distances in females, mainly because of lower ENK mRNA and higher running distances characteristic of HCR.

Striatal enkephalinergic differences between HCR and LCR rats support two other putative mechanisms of motivated behavior in rodents. First, differential ENK expression is consistent with previous evidence that dopaminergic transmission to the striatum contributes to differences in locomotory behavior (Mathes et al., 2010; Wise, 2004), possibly via opioidergic regulation (Roberts et al., 2013). It is plausible that greater dopaminergic tone suppresses transcription of ENK in HCR (Foley et al., 2006; Li et al., 1990; Young et al., 1986) promoting wheel-running and other appetitive behaviors related to energy balance (Borer, 2010; Smith et al., 2011). Second, it has been theorized that ENK innervation from the dorsal striatum drives motivation specific to food seeking (Salamone et al., 2003) and reward (Hayward and Low, 2007). The tendency toward less dorsal striatal ENK expression

observed here in HCR compared to LCR suggests a novel, central mechanism underlying reduced sensitivity of HCR to an obesogenic environment compared to LCR rats (Koch et al., 2012; Novak et al., 2010).

Our findings encourage investigation of whether decreased transmission (lower ENK) or increased transmission (upregulated opioid receptors) is associated with wheel running. The complex interactions between opiodergic and dopaminergic pathways through the basal ganglia require further study to identify an integrated neural mechanism underlying voluntary locomotory behavior.

4. Experimental Procedures

4.1. Animals and Experimental Design

40 adult Female rats (n=20 HCR, n=20 LCR) and 40 adult Male rats (n=20 HCR, n=20 LCR) were blocked by sex and line before random allocation (www.randomizer.org) to activity wheel (n=40) or sedentary (n=40) conditions. Animals were housed individually in polycarbonate cages in a temperature and humidity-controlled environment on a 12-hour light/dark schedule. Food and water were available ad libitum and animals were weighed upon housing assignment and prior to decapitation. Selectively bred HCR and LCR rats were obtained from the University of Michigan where the running capacities were estimated by treadmill tests performed at 11 weeks of age (Table 1). All procedures were approved by the institutional animal use committees at the University of Georgia and the University of Michigan and conducted in accordance with NIH Guide for Care and Use of Laboratory Animals.

4.2 Exercise Protocol

Activity wheels with a circumference of 105 cm were placed in polycarbonate shoebox cages and attached to magnetic revolution counters (MiniMitter; Bend, Oregon). Home cages of sedentary rats did not contain an activity wheel. Activity-wheel running rats were given unlimited access to activity wheels for 21 days. Wheel revolutions were recorded and daily distances were determined by multiplying the circumference (105 cm) of the activity wheel by the number of revolutions.

4.3 In Situ Hybridization Histochemistry

Animals were killed by rapid decapitation upon termination of the 21 day exercise or control exposure at the end of a full light cycle. Brains were extracted and stored at -80°C . Brains were sliced into 12 μm coronal sections at the level of the nucleus accumbens and thaw-mounted to gelatin coated microscope slides. Anatomical location was verified using a 0.1% thionin stain. In situ hybridization methods used are reported in detail elsewhere (Van Hoomissen et al., 2003). Briefly, sections were fixed in 4% (v/v) formaldehyde in 0.12 M sodium phosphate-buffered saline (PBS) solution, rinsed in PBS, and soaked in 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine HCl-0.9% (v/v) NaCl. Sections were then dehydrated through a series of ethanol washes, delipidated in chloroform, rinsed again in ethanol, and allowed to dry.

An oligonucleotide probe complementary to prepro-ENK mRNA was obtained from Oligos, Etc. (Wilsonville, OR) and labeled at the 3' end with [35S]-dATP (New England Nuclear, Boston, Massachusetts), terminal deoxynucleotidyl transferase (TdT, 25 units/ml; Roche, Indianapolis, Indiana), and tailing buffer. Agilent (Santa Clara, CA) NucTrap size exclusionary columns were used to separate unincorporated nucleotides from labeled ENK. Sections were hybridized with the radiolabeled ENK in solutions containing 25% (v/v) formamide, 72 mM NaCl, 3.2 mM Tris-HCl, .0032 mM EDTA, 0.001% (v/v) sodium pyrophosphate, 0.004% (v/v) sodium dodecyl sulfate, 0.002 mg/ml heparin sulfate, and 2% (v/v) dextran sulfate. Hybridized sections were incubated overnight at 37°C, followed by a series of washes in SSC and SSC-50% formamide, water and ethanol to reduce nonspecific binding, and then were dried. Hybridized brain sections were exposed, with a radioactive standard, to autoradiographic film for four weeks.

Developed autoradiographic film was photographed (Nikon D5000) and analysis was conducted, blind to assignment and line, using NIH ImageJ (version 1.44). Gray-scale was converted to μCi values using the radioactive standard and calibration was performed for each film. Measurements were taken at points in the left and right ventral (NAS and OT) and dorsal striatum and compared to background recorded for each slide. Outliers were omitted within each site across all slides using Grubbs Criteria (Grubbs and Beck, 1972), and as a result one HCR female was removed from analysis of ENK expression in the OT. There was moderate-to-high agreement (ICC (2, 4)) among females (Cronbach α = .833, .886, and .819) and males (Cronbach α = .774, .658, and .849) for ENK in the OT, NAS, and dorsal striatum respectively.

4.4 Data Analysis

Data were analyzed using SPSS Windows version 21.0 (IBM Corporation, USA). All significance levels were set at $p < 0.05$. For descriptive purposes, distance run was analyzed by a 2 (line: HCR vs. LCR) $\times$ 3 (time: weeks 1–3) mixed-model ANOVA with time repeated. Body weight was analyzed using a two-way (condition $\times$ time) mixed model ANOVA with time repeated. Huynh-Feldt (ϵ) was used to adjust degrees of freedom (df) for sphericity violation of the repeated measure. For hypothesis testing, levels (μCi) of ENK mRNA in each brain region were compared using a 2 group (HCR vs. LCR) $\times$ 2 condition (activity wheel vs. sedentary) ANOVA. Bonferroni-adjusted post-hoc tests were used. Effect sizes were estimated by η^2 . Relations between ENK mRNA and running distance were estimated by linear regression analysis. The sample size was sufficiently powered to detect moderately large effect sizes ($\eta^2 \geq .15$) at a statistical power $> .80$, and $p < .05$.

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Research Highlights

- ENK mRNA in ventral striatum was lower in a divergent line of high running rats.
- Exposure to wheel running did not alter striatal ENK.
- Motivational drive to run is plausibly mediated by intrinsic striatal ENK.

Fig. 1. Mean daily running distances ($\pm$ SEM) on the activity-wheel over 3 weeks. High-capacity running (HCR) rats ran more on average than low-capacity running (LCR) rats. There was an interaction effect between lines over 3 weeks in females; the effect was independent of line in males.

a

b

Fig. 2.

(a) Average enkephalin (ENK) mRNA expression ($\pm$ SEM) in the nucleus accumbens septi (NAS). High capacity running (HCR) rats expressed less ENK than low capacity running (LCR) rats; OT=olfactory tubercle, NAS= nucleus accumbens septi. (b) Average enkephalin (ENK) mRNA expression ($\pm$ SEM) in the dorsal striatum. There were no significant line differences in ENK expression.

Fig. 3.

Mean daily running distance and ENK mRNA expression among female rats in the nucleus accumbens septi (NAS; panel A) and the olfactory tubercle (OT; panel B). Correlations between running distance and ENK mRNA in the NAS and the OT were mainly explained by lower mean mRNA and higher mean running distance in HCR females compared to LCR females.