EXPRESSION OF MESSENGER RNA (mRNA) FOR BRAIN DOPAMINE RECEPTORS AND ENKEPHALIN BEFORE AND AFTER WHEEL RUNNING IN RATS SELECTIVELY BRED FOR HIGH- AND LOW- AEROBIC CAPACITY

By

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(Under the Direction of Rodney K. Dishman)

ABSTRACT

We examined whether female rats selectively bred for high intrinsic aerobic capacity (High Capacity Runners, HCR) and low intrinsic aerobic capacity (Low Capacity Runners, LCR) differentially express mRNA in the dopaminergic and opiodergic pathways of the basal ganglia and that 3 weeks of access to activity wheels alters expression in those pathways. Consistent with predicted outcomes, enkephalin (ENK) was expressed at lower levels in the nucleus accumbens septi (NAS) and olfactory tubercle (OT) in HCR females compared to LCR females. D_1 receptor mRNA expression in the dorsal striatum and NAS was decreased in rats with running wheel access compared to those that were sedentary for 3 weeks. Contrary to predictions, tyrosine hydroxylase (TH) and D_2 mRNA expressions were not significantly different between HCR and LCR strains and were not affected by wheel running. The results suggest that female rats selectively bred for intrinsically high aerobic capacity demonstrate a high level of voluntary physical activity that is associated with high dopaminergic tone, consistent with low ENK mRNA expression in the basal ganglia. Increased voluntary wheel running results in downregulation of D_1 transcription in the ventral striatum regardless of intrinsic running capacity.

INDEX WORDS: activity wheel running, *in situ* hybridization, opioids, mesolimbic dopamine pathway

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CHAPTER 1

INTRODUCTION

A growing body of evidence supports dose-dependent decreases in the risks of several chronic diseases and early mortality among people who are physically active (Physical Activity Guidelines Advisory Committee, 2008; Warburton, Nicol, & Bredin, 2006). Additionally, models of physical activity using rodents and primates have shown that voluntary physical activity and exercise training favorably influence neurogenerative, neuroadaptive, and neuroprotective processes in the brain and spinal cord (Cotman & Berchtold, 2002; Van Praag, 2008) that have implications for the prevention and treatment of major depression, some anxiety and sleep disorders, the decline in cognition associated with aging, and neurological disorders such as Parkinson's disease, Alzheimer's dementia, multiple sclerosis, ischemic stroke, and head and spinal cord injury (Dishman et al., 2006; Physical Activity Guidelines Advisory Committee, 2008).

Despite its importance for public health, the physical activity of many U.S. adults is below recommended levels (U.S. Department of Health and Human Services, 2008). Although 3 of 4 adults report participation in some form of leisure-time physical activity during the past month (Centers for Disease Control and Prevention, 2005), less than half report frequencies corresponding with currently recommended levels of moderate or vigorous physical activity, and necessary to maintain fitness and promote health (Centers for Disease Control and Prevention, 2007; Barnes, 2007).

Growing evidence from family and twin studies suggests that variation in human physical activity has a substantial genetic basis (Perusse et al., 1989; Stubbe et al., 2006) that is poorly understood (Dishman, 2008; Hagberg et al., 2011; Rankinen et al., 2010). Wheel running by rodents provides a model for understanding the genetic basis of voluntary physical activity (Lightfoot, Hamilton & Moore-Harrison, 2010). Examination of the rat and mouse genomes indicates similar variability of a gene and physical activity interaction, while genetic and phenotypic manipulation achievable in rodents produces further evidence of a genetic predisposition to participation in daily physical activity (Kelly et al., 2010; Knab & Lightfoot, 2010; Lightfoot, Turner, Daves, Vordermark, & Kleeberger, 2004; Lightfoot, Turner, Pomp, Kleeberger, & Leamy, 2008; Swallow, Carter, & Garland, 1998). A recent review of 12 studies, using human and mouse gene modeling, found that genetics accounts for 20-92% of the physical activity performed (Lightfoot, 2011b); these findings are similar to results of family studies of both monozygotic and dizygotic twins which have found that 20%-70% of variation in human physical activity is inherited (Carlsson, Andersson, Lichtenstein, Michaelsson, & Ahlbom, 2006; Eriksson, Rasmussen, & Tynelius, 2006; Lauderdale et al., 1997; Stubbe et al., 2006).

Investigations of the common loci associated with high levels of daily physical activity have provided highly varied results most of which disagree on specific sites of importance (De Moor et al., 2007; Hagberg, 2010; Simonen et al., 2003). A few association and linkage studies have implicated candidate genes that might explain small, but significant, portions of the genetic variation in physical activity. Many of those genes represent an influence on energy intake pathways (e.g. *LEPR, AGRP, MC4R*), while only a handful support a model of motivated physical activity (e.g. *DRD1, DRD2, DRD4, SERT, 5-HT2A, 5-HT2C, Orexin A*) (Dishman,

2008). Theories implicating dysregulated energy balance in sedentary behavior and obesity are well substantiated (Donahoo, Levine, Melanson, 2004; Mustelin, et al., 2009; Ravussin & Bogardus, 2000), but few studies have examined the etiology of intrinsic motivation or the impact that brain transcriptional differences have on voluntary physical activity.

Pharmacological evidence supports a putative role of dopamine receptors in the regulation of physical activity (Knab, Bowen, Hamilton, Gulledge, & Lightfoot, 2009; Rhodes & Garland, 2003). In particular, dopaminergic medium-spiny neurons linking the basal ganglia and ventral tegmental area (VTA) may be important in describing wheel running as a motivated behavior. The VTA, a primary source of dopamine (DA), has excitatory control over the basal ganglia via a high concentration of D_1 and D_2 receptos in the striatum, but is inhibited by DA autoreceptors necessary to prevent excitatory toxicity (Cooper, 2002). The basal ganglia integrate signals from the prefrontal cortex and amygdala, and in turn have inhibitory control over the VTA via GABAergic neurons (Van Bockstaele & Pickel, 1995). Werme et al. (2002b) suggest that gene expression in striatal opioid pathways promotes addictive behavior, including those driven by natural reward, such as the drive to exercise (Nestler, Barrot, & Self, 2001; Werme et al., 2002). Furthermore, midbrain dopaminergic pathways through the basal ganglia are influenced by opioids, dynorphin (DYN) and enkephalin (ENK), also found to be influenced by physical activity (Blake, Stein & Vomachka, 1984; Chen, Zhao, Yue & Wang, 2007; Dishman & O'Connor, 2009), and commonly accepted to influence hedonic, reward, and pain signaling.

By studying the selectively bred line of high- and low-capacity running rats developed at the University of Michigan, it is possible to investigate the influence of gene-environment

interactions on physical activity. Eleven generations produced a 347% difference in running capacity, 450% difference in aerobic capacity on a treadmill, and between 24% and 39% difference in body weight between the high- and low- capacity runners (Koch & Britton, 2008a). Past research shows that divergent running behaviors result in a wide range of physiological differences, due in part to the 1,540 genes expressed differently between the two lines (Bye et al., 2008). The physiological and morphological differences of the high- and low-running rats have been thoroughly described, but only a handful of studies attempt to describe adaptive differences in the central nervous system (Foley et al., 2006; Foley, Greenwood, Koch, Britton, & Fleshner , 2005; Waters et al., 2008). There is strong evidence for the effects of physical activity on brain neurotrophins such as brain derived neurotrophic factor and galanin (Cotman & Berchtold, 2002; Holmes, Yoo & Dishman, 2006; Neeper, Gomez-Pinilla, Choi & Cotman, 1995), monoamines including norepinephrine (Dunn et al., 1996) and serotonin (Dunn , Trevedi & O'Neal, 2001), and also on ENK, DYN and DA—three primary signaling molecules of the motor-limbic pathways (Foley, Greenwood, Koch, Britton, & Fleshner , 2005; Johnson & Mitchell, 2003; Waters et al., 2008).

The purpose of this study was to evaluate the differences in DA, ENK and DYN mRNA expression in the VTA and NAS of animals selectively bred for running behavior, and the possible influence of voluntary exercise on monoamine and neuropeptide mRNA expression. Previous investigations of midbrain DA and basal ganglia interaction have not considered intrinsic differences that may exist between strains bred for aerobic capacity, while those studies that have sought to outline strain differences have tended to use inbred mouse strains and broad spectrum quantitative trait loci (QTL) analysis (Lightfoot et al., 2007; Lightfoot et al., 2010) or

lack a central unifying mechanism to explain running as a motivated behavior (Rhodes, Gammie & Garland, 2005). The aims of the following review and experimental report are to demonstrate plausibility for the hypothesis that voluntary physical activity has a genetic basis that involves intrinsic features of dopaminergic-opiodergic regulation of the basal ganglia that are also adaptive in response to chronic running exposure.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

In literature reporting on human and animal voluntary behavior, ranging from attraction to drug addiction, there is implicit interest in the psychobiological precursors of motivation. Decades of data conclusively suggest that areas most likely to have a role in these processes are the mesocorticolimbic dopaminergic pathways to the striatum, and the numerous pathways, expressing a wide range of neuropeptides and monoamines, projecting from the striatum (Bozarth & Wise, 1986; Koob, 1992; Phillips & LePiane, 1980). Moreover, the surrounding basal ganglia, contain high concentrations of receptors for the endogenous opioids ENK and DYN known to mitigate mood, pain, and pleasure experience (Hawkes, 1992; Leknes & Tracey, 2008, Mansour, Fox, Akil & Watson, 1995, Nieto et al., 2005), and are also well documented as part of a limbic-motor integration circuit, specifically the ventral pallidum and amygdala (Haber & Watson, 1985; Koepp et al., 2009). There is a heavy concentration of dopaminergic neurons between the VTA and basal ganglia, specifically the striatum, a region marked by high D_1 and D_2 receptor concentration expressed on ENK and DYN neurons (Gerfen et al., 1990). It is generally accepted that DA drives the 'wanting' of a rewarding stimulus, but is not necessarily responsible for the hedonic appraisal, or 'liking' of the stimulus (Berridge, 1998; Smith & Berridge, 2011). Collectively this suggests a possible interaction between the opioidergic and dopaminergic pathways in response to physical activity, which could result in altered motivation to exercise.

Likewise, *DRD1* and *DRD2*, genes for D_1 and D_2 receptors expressed in the striatum, are part of a handful of candidate genes found to be differentially expressed between strains of rodents that also differ in levels of voluntary wheel running (Dishman, 2008). It is plausible then that TH mRNA, reflecting altered DA levels, and ENK and DYN mRNA, opioids produced in neurons co-expressing D_1 and D_2, all may be differentially expressed between selectively bred strains and as a result of physical activity. LCR and HCR rats are selectively outbred based on aerobic capacity, previously shown to positively correlate with distances run on an activity wheel (Murray et al., 2010), and therefore provide a reliable model by which to study the effects of intrinsic differences in aerobic capacity on mRNA expression using a factorial experimental design.

Opioid Response to Exercise

Endogenous opioids (EO) are neuropeptides that behave as ligands, are internally derived, and bind to selective receptors in the brain found to modulate perception of pain and pleasure in response to a variety of stressors (Leknes & Tracey, 2008). There are three major 'families' of opioid peptides: enkephalins, dynorphins and endorphins. The bulk of current research is concerned with β-endorphin, but, as only a small part of the system, is concentrated around cortical areas controlling autonomic function, such as the arcuate nucleus, hypothalamus, and the brainstem (Jonsdottir, 2000). The release of endorphins from the pituitary into the bloodstream, well documented during exercise, causes release into the peripheral nervous system. Fifty-nine of 65 studies from 1982 to 2008 show significant increases in peripheral concentrations of β-endorphins as a result of exercise (Boecker et al., 2010).

Animal models have historically used cerebrospinal fluid concentrations of opioid metabolites to approximate activity in the brain, but origin of the opioids cannot be determined using this measure—a confound since opioids are produced systemically as well as centrally (Hoffmann, Terenius and Thorén, 1990; Jonsdottir, 2000). To compound this problem, passage across the blood brain barrier is size selective thereby blocking entry of opioids formed in the periphery and re-entry of those produced in the ventricles. A recent study of German males, a PET study by Boecker et al. (2008), was the first to show exercise-associate endorphin release in frontal limbic areas implicated in emotional processing, such as the anterior cingulated cortex (ACC). The impact of these findings could be eclipsed by flaws that are found in most endorphin-hypothesis models—most notably a lack of reliable and valid data showing that self-reports of euphoria are correlated to endorphin release (Dishman & O'Connor, 2009).

While consensus previously indicated 80% VO_2max as the threshold for significant acute changes in circulating β-endorphin concentrations, research over the past 30 years has produced conflicting theories for dose-response effects of exercise on both endorphinergic precursors, adrenocorticotrophic horomone (ACTH) and proopiomelanocortin (POMC), and serum concentrations the neuropeptide (Harbach & Hempelmann, 2005; Nybo & Secher, 2004). Research also suggests that peripheral changes are not reflective of β-endorphin concentrations in the brain, and that while release into the periphery may alter sensory feedback, specifically pain signaling, during exercise, it is unlikely that mood or psychophysiolgoical state is affected via the same mechanism (Boecker et al., 2010; Nybo & Secher, 2004; Rossier, et al., 1977). While this suggests a wide range of possible systemic outcomes, current evidence reflects early findings that the two non-POMC derived opioids, ENK and DYN, are ligands with greater

cerebral effects due to their proximity to areas of motor control and monoamine transmission (Fallon, 1986; Foley & Fleshner, 2008;).

The interface of the basal ganglia and mesolimbic dopamine system is marked by high concentrations of ENK and DYN expressing neurons (Robbins & Everitt, 1996; Spanagel, Herz & Shippenberg, 1992). Activity in these brain areas are implicated as centers for hedonic signaling associated with motivation and reward, and responsible for controlling behavior including feeding and locomotion (Lutter & Nestler, 2009; Wang, Volkow, Thanos, & Fowler, 2009;). Many effects of exercise, such as hyperphagia (Davis, Lamb, Lowy, Yim, & Malven, 1985), increased natural killer cell activity (Fiatarone et al., 1988), and increased place preference for morphine (Eisenstein, 2007), can be linked to concomitant increases in opioids. Likewise, there is considerable evidence demonstrating that physical activity has effects on expression of opioidergic mRNA, but little agreement on the direction or magnitude of these effects (Bjørnebekk, Mathé, & Brené, 2005a; Bjørnebekk, Mathé, & Brené, 2005b; Siegel et al, 2009).

DYN is part of a larger pathway responsible for behavioral manifestations of energy imbalance such as activity-induced anorexia and obesity, and additional research could yield a much larger scale interaction between reward, hunger, and motor pathways (Koob & Le Moal, 2008). There is strong evidence that DYN release in the paraventricular nucleus, as a result of aerobic exercise, is associated with release of other neuropeptides associated with energy balance, such as neuropeptide-Y (Chen, Zhao, Yue, & Wang, 2007), and that the neuronal subpopulations expressing DYN and D_1 receptors are also affected (Gerfen, 2000). Furthermore, opioid release in the paraventricular nucleus directly affects the release of DA and acetylcholine

in the NAS, and therefore preference for food, ethanol and potentially running behavior (Rada, Barson, Leibowitz, & Hoebel, 2009).

DYN exists as either $DYN_{A\ 1\text{-}17}$, $DYN_{B\ 1\text{-}13}$, or α-neo-endorphin, all of which are cleaved from prodynorphin by proprotein convertase 2, and bind to kappa-opioid receptors (KOR) (Day et al., 1998). Activation of KOR in the dorsal raphe nucleus causes stress induced dysphoria and aversion as a result of serotonergic projections to the NAS (Land et al., 2009). DYN, not unlike its opioid counterparts, is found centrally in the amygdala and periaqueductal grey, and in the dorsal horn of the spinal cord, and it has both central and widespread peripheral effects (Jonsdottir, 2000). One pathway possibly activated by high-intensity physical activity is initiated by DYN-A release in the spinal cord causes blood vessel dilation, and results in hyperalgesia via bradykinin receptor activation (Luo et al., 2008).

Cocaine and wheel running cause an increase in DYN-A mRNA in the caudate putamen, part of the basal ganglia central to learning and locomotion, in rats bred for drug and running preference—running does not have the same effect in rats bred without the same preference indicating a genetic influence and a putative mechanism by which these regions reinforcement and motivation for physical activity (Nestler et al., 2001; Werme et al., 2000).

Neuropeptides in the ENK family come in two forms: leu-enkephalin, coded by the enkephalin and dynorphin genes, or met-enkephalin, coded by the enkephalin and preopiomelanocortin gene. ENK binds to delta-opioid receptors (DOR), have anti-depressant and anxiolytic effects, and also cause upregulation of BDNF (Perrine, Sheikh, Nwaneshiudu, Schroeder, & Unterwald, 2008; Torregrossa et al., 2006).

It is possible that ENK has effects on higher brain function, and may be integral in the systemic stress response to exercise. Recent findings published by Siegel et al. (2009) have yielded a theory by which inhibitory enkephalinergic projections to the LC, activated by wheel running, not only decrease central norepinepherine release, but also attenuate its stress-induced release from the spleen. It is possible that physically active individuals, compared to sedentary individuals, mitigate the activity of intense stressors via greater activation of inhibitory ENK pathways thereby better constraining sympathetic nervous system response. Evidence suggests that D_2 receptors co-exist on striatal ENK projections, and terminate in the ventral pallidum, an area also associated with regulation of motivated locomotor behavior (Lu, Behnam, Ghasemzadeh, & Kalivas, 1997).

The mesolimbic dopaminergic junction in the opioidergic neurons of the striatum is important for locomotion, but the primary neurotransmitters are not solely responsible for regulation of these pathways. Immediate early genes are activated rapidly, within minutes of onset of a triggering stimulus, activation can last exponentially longer, but doesn't require the formation of proteins in order to undergo transcription. Since these changes are not as complex as molecular adaptations that occur downstream, it is easier to associate immediate early gene response with phenotypical changes. Effects of exercise tied to immediate early gene expression, such as those from *fos* and *jun* family (Puntschart et al., 1998), include altered immune response (Simon, Fehrenbach & Niess, 2006) and hippocampal plasticity (Tong, Shen, Perreau, Balazs & Cotman, 2001). Werme et al. (2002b) illustrated the effects of exercise on the opioidergic pathways by finding early-gene upregulation occuring in DYN neurons, compared to ENK neurons, in animals with high levels of physical activity physical activity which may occur

as a results of activity-induced increases in DA transmission, as measured by TH (Greenwood et al., 2010). It is probable then that DYN and ENK have opposing mechanisms in the feedback pathways from the striatum to the VTA controlling DA release.

The effects of these neuropeptides are rooted in systemic inflammatory responses. It is possible that an individual's subjective exercise experience is directly related to a balance between these endogenous opioids, centrally and peripherally, and the effects of DA pathway activation in the corticolimbic system (Dishman & O'Connor, 2009). While endogenous opioids play an integral role in signaling between many regions of the brain concerned with movement, learning, motivation and reward, it is plausible that the resulting behavioral and morphological changes can be traced back to DA mediated activation of the basal ganglia.

Dopaminergic response to exercise

The neurobiological precursors for motivation have been studied for decades in the hopes that we can maximize our individual potential, and possibly redefine the boundaries of 'free-will'. The Dopamine Hypothesis, one of the benchmark theories in the history of neuroscience, indicates that the namesake neurotransmitter is the keystone to reinforcement and reward circuitry in the brain. Reinforcement is better described as a retroactive effect on learning, since it is a series of events that occurs only after the behavior is learned (Wise, 2004). The neurotransmitters invoking this reinforcement increase the probability that the behavior will be repeated. Originally a very broad theorem, this hypothesis is rooted in early work by Thorndike, who coined "The Law of Effect" and argued that responses which bring feelings of satisfaction or pleasure to the animal are more likely to be repeated as a result of a strong connection between the animal and associated situation which brought on positive feelings (Salamone &

Correa, 2002). Skinner later updated the language of the theory to allow for use in the modern field; 'reinforcers' replaced 'satisfiers', but the basic concepts remain.

Dopamine has two main functions in the central nervous system: it acts both as an inhibitory neurohormone, suppressing release of prolactin, and as a monoamine neurotransmitter, widely produced across the brain, activating any of the five isoforms of the dopamine receptor. There are five distinct types of dopamine receptors (D_1-D_5), (Missale, Nash, Rombinson, Jaber, & Caron, 1998). More recently they have been re-grouped as D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors each having different functional and behavioral implications depending on the brain region where they are localized. D_1-like receptors synapse on neural membranes and veins, and are positive regulators of protein kinase A, through G-protein activation of cAMP, resulting in neurotransmitter release. D_2-like receptors synapse on neural membranes and glia, and have the opposite effect on cAMP (Choi, Chen, Hamel, & Jenkins, 2006; Vallone et al., 2000). Most research of the past few decades concerns DA activity as a dependent measure of stimulation (i.e. in response to drug administration) or as an independent factor regulating motivation to seek out rewarding stimuli, but the mechanism by which DA is involved in voluntary physical activity is unclear.

It is possible that DA works via altered thermoregulation in a post-exercise condition (Brown, Bae, & Kiyatkin, 2007). For instance, decreased dopamine receptor activation and binding could decrease tolerance to changes in body temperature (Balthazar, Leite, Ribeiro, Soares, & Coimbra, 2010). This would result in improper heat dissipation during recovery, and possibly create aversive association with exercise in those previously predisposed to avoid physical activity. Another theory, by Knutson & Gibbs (2007), suggests that D_1 receptors are

responsible for exercise-related reward, and that blood oxygen level dependent signaling is the conduit for such a pathway, but the theory hasn't been corroborated. It is more likely that voluntary physical activity operates through a mechanism similar to that of cocaine, amphetamines, alcohol, and in some cases, food, water and sucrose.

Early findings by Ungerstedt and Arbuthnott (1970) demonstrated that lesions in these midbrain DA pathways result in deficits specific to motivated locomotor behavior (Wise, 2004). Animals will not lever-press for positive stimuli such as sexual contact, food, or even water if these DA pathways are inhibited (Wise & Schwartz, 1981). Conversely, data suggests that locomotor tasks cause increased DA concentrations in the dorsal striatum (Meeusen & De Meirleir, 1995; Ouchi et al., 2002), and increases in this region have previously been shown as a result of systemic stress response, specifically corticosterone release which also occurs during exercise (Heyes, Garnett & Coates, 1988; Rougé-Pont, Deroche, Moal & Piazza, 1998). Amphetamine administration to the NAS increases responding to stimuli previously paired with hedonic flavors, such as sucrose, in part through increased D_1 and D_2 activation (Robbins, Cador, Taylor, & Everitt, 1989). A study of wheel-running paired with cocaine self-administration suggest the two are substitutable as reinforcers—when offered one freely, rats will decrease behavior associated with the other (Cosgrove, Hunter & Carroll, 2002) and reflects the finding that wheel running itself is rewarding (Brené et al., 2007). Additionally, D_1 agonists increase locomotion, whereas D_2 agonists reduce locomotor function suggesting different feedback pathways from the striatum to the VTA (Jackson & Westlind-Danielsson, 1994). Speculation exists concerning the exact mechanism by which these receptors act in response to physical

activity, or to regulate it as an independently motivated behavior, and not as an intermediate behavior to obtain external reward.

A wealth of evidence has described the integral role the basal ganglia plays in locomotion (Garcia-Rill, 1986; Garcia-Rill, Skinner & Fitzgerald, 1985; Jahn et al., 2004; Marsden & Obeso, 1994; Smith, Feldman & Schmidt, 1988, Takakusaki, Oohinata-Sugimoto, Saitoh & Habaguchi, 2004). Constituted by the globus pallidus and striatum on the caudal border, and rostrally by the subthalamic nucleus and substantia nigra, the basal nuclei results in direct and indirect efferent pathways influencing locomotor activity (Parent & Hazrati, 1995). Cortico-thalamic neuronal loops, necessary for sensori-motor processing are integrated through the basal ganglia where release of excitatory (i.e. dopamine and glutamate) and inhibitory (i.e. GABA and serotonin) neurotransmitters influence firing activity (Herrero, Barcia & Navarro, 2002).

Specifically dopaminergic signaling from the midbrain to distinct areas of the ganglia, specifically the striatum, have received significant attention controlling motivated behavior (Cardinal, Parkinson, Hall & Everitt, 2002; Depue & Collins, 1999; Horvitz, 2002;). Evidence suggests that motor cortex activation is driven by dopaminergic projections from the VTA and substantia nigra, which synapse on GABAergic and opioidergic neurons in the ventral striatum—comprised of the NAS and olfactory tubercle (OT) (Churchill, Klitenick & Kalivas, 1998; Graybiel, 1990), but the ventral pallidum, another signal integration center, plays a regulatory role in locomotion as well and emphasizes the complexity and scope of this network (Dishman et al., 1996; Johnson & Stellar, 1994)

From an evolutionary perspective, these pathways have been suggested to maintain homeostasis during famine and starvation by increasing locomotive behavior to obtain food

(Lenard & Berthoud, 2008), or to find alternative sources of food when the energy demands to obtain a preferred source become too great (Salamone, Correa, Mingote, & Weber, 2003). Moreover, the dysregulation of glutamate and DA in this system, as a result of neuronal death, has been implicated in locomotor disruption found in heritable, degenerative diseases such as Parkinson's (Carlsson & Carlsson, 1990). The neurobiology of motivated wheel running is as yet unknown, but it is plausibly manifest as a result of interactions between multiple signaling pathways in the mesolimbic-motor interface driven by DA from the VTA (Burgess, 2010; Knab, Bowen, Hamilton, Gulledge, & Lightfoot, 2009; Scheurink, Boersma, Negardh, and Sodersten, 2010).

The VTA in the midbrain serves as center of dopamine release for the entire forebrain, including limbic and motor regions (Ikemoto, 2007). These pathways also synapse back onto the VTA to create a feedback 'limbic loop'. The importance of the VTA may lie in the presynaptic D_2 receptors that are found on glutamatergic neurons; the activation of these receptors inhibit the excitatory signaling of glutamate which in turn decreases excitability and alters firing patterns of dopaminergic neurons (Koga & Momiyama, 2000). An exercise induced increase in DA concentrations could change the striatal firing pattern thereby eliciting motivation to engage in physical activity.

Most research indicates that species-specific differences exist in the sub-structure of the VTA, but cytoarchitectural evidence combined with neurochemical analysis produced a common four-region model in the rat: paranigral nucleus, parabrachial pigmented area, parafasciculus retroflexus area, and the ventral tegmental tail (Halliday & Tork, 1986; McRitchie et al., 1996,). The middle two-thirds of the VTA, comprised of the paranigral nucleus and parabrachial

pigmented area, contains the highest concentrations of TH positive cells (Ikemoto, 2007; Phillipson et al., 1979). The neurons from this region that project to the striatum are considered part of the mesocorticolimbic DA pathway, an important distinction from neurons that originate from the contiguous nigral region forming the nigrostriatal DA pathway (Wise, 2009).

The striatum, encompassing the shell and core of the NAS, and lateral and medial olfactory tubercle (OT), contain heavy concentrations of both D_1 and D_2 receptors expressed on ENK and DYN neurons (Ikemoto, Glazier, Murphy, & McBride, 1997). Efferents from the shell innervate the VTA, and continue on to the mesocorticolimbic structures whereas the core is more closely tied to striatal activation (Deutch & Cameron, 1992). The deactivation of both core and shell decrease reinforced responding, but the core controls the intensity of response via the limbic-motor connection. This is specific toward a directed, exteroceptive goal whereas the shell is more important for goals driven by internal cues (Di Ciano, Robbins, & Everitt, 2007). Both of these could be important for motivation to exercise, since humans are influenced by cues both external (i.e. media, people, cost, body image) and internal (i.e. feelings of fatigue and energy, health, hydration) when making the decision to be physically active.

All evidence suggests physical activity modulates the DA pathways from the VTA to the striatum, but the study of DA independently regulating physical activity have yet to yield conclusive results (Knab & Lightfoot, 2009). Due to the particularly complex nature of signaling in the limbic-motor regions of the midbrain and forebrain, progress toward a unifying theory of DA action on levels of physical activity requires a comprehensive model of pathway influences in the region with the primary goal to describe voluntary physical activity as a unique motivated behavior.

Heritability of Physical Activity

The extent of heritability of physical activity is still unclear, with estimates for genetic influence on obesity ranging from 20-70% in multiple twin and familial studies (Bray et al., 2009; Dishman, 2008; Perusse, Tremblay, Leblanc, and Bouchard, 1989; Rankinen et al., 2010; Stubbe et al. 2006;). The human study reporting the highest genetic influence on levels of physical activity is also the only study to date to directly, and quantitatively, measure physical activity by combining doubly-labeled water and accelerometry data in twins (Joosen et al., 2005)

Overall, there have been 221 autosomal and x-linked genes associated with phenotypes expressing differing levels of physical activity, and 119 QTL related to physical activity- linked traits (Bray et al., 2009). Genetic influences on physical activity most likely have effects on two levels. First, it is possible that candidate genes controlling energy pathways, such as uncoupling protein 2 (UCP2) and angiotensin precursor (AGT M235T), act on physical activity through modulation of energy balance (Bouchard, Perusse, Chagnon, Warden, & Ricquier, 1997; Fleury et al., 1997; Bray, et al., 2009). Second, another group of candidate genes directly control reward and motivational pathways, such as precursors for dopamine, serotonin, norepinephrine and their associated receptors, but have not been linked to differences in physical activity (Dishman, 2008). Evidence suggests that dopamine, serotonin, and norepinephrine transporter gene expression is linked to the rewarding effects of cocaine (Sora et al., 2001) and the deleterious effects of depression (Perona et al., 2008). Considering the role DA plays in locomotion, the mesolimbic DA pathway between the VTA and the striatum is a logical region in which to examine gene expression relating to these molecules (Alex & Pehek, 2007). There are currently only two candidate genes that are supported by four independent analyses and

techniques to regulate physical activity levels: *DRD1,* discussed previously, and *Nhlh2*, shown to regulate conversion of two hormone conversion enzymes (PC1 and PC2). It is also clear that while haplotype difference and QTL studies are at a surplus, candidate genes lacking support require corroboration using gene-expression techniques (Lightfoot, 2011a).

Increasingly, research has depended on the use of non-primate animals, especially rodents such as mice and rats, to further our understanding of the influence of candidate genes. One way to provide more accurate models of human behavior is through selective breeding, which recognizes that phenotypic and genotypic differences can be bred for over multiple generations. The most successful rodent models of human physical activity, as an innate behavior, utilize rats and mice bred based on running capacity, either voluntary (activity-wheel) or forced (treadmill) (Koch & Britton, 2001; Swallow, et al., 1998).

The transgenic knock-out (KO) method, typically performed on mice, is one that has produced highly significant findings. By breeding mice with genetically altered dopamine production, or expression of associated receptors, studies have demonstrated the importance of dopamine in both consolidation of wheel running behavior (Vargas-Pérez, Borrelli, & Díaz, 2004) and related deficits in locomotor activity in accumbens D_2 receptor knock-outs (Tran et al., 2002). Recent studies documenting rodent stress response show promise for a possible difference in running-mediated adaptation in the reward pathway. In post-exercise analysis, there are significant differences in plasma corticosterone and striatal dopaminergic concentrations (Waters et al., 2008). Furthermore, mice bred with heterozygous deletion of *Nurr1*, a gene that codes for a transcription factor in DA cells in the ventral midbrain, never

develop a preference for alcohol, nor do they display high levels of wheel running (Werme et al., 2003).

With the exception of conditional KO models, which allow for genetic code to be disabled after birth, many KO models can be confounded by adaptation across the lifespan of the animal. Receptors, neurons and entire areas of the brain can, at least partially, functionally replace the missing genes. Breeding by phenotype allows researchers to attain separate lines of animals significantly different in a certain characteristic, while maintaining genetic heterogeneity in other areas of the genome. Widely used lines bred for high- and low-running capacity differ on many physiological measures such as metabolic recovery (Torvinen et al., 2010), peripheral oxygen transportation (Howlett et al., 2009), and VO_2max (Gonzalez et al., 2006b). Additionally, a recent study found that two lines of rats, one bred for exercise and one bred for obesity, also had very different dopaminergic activity in the mesolimbic structures, and both lines showed dopaminergic dysregulation compared to a wild-type control, supporting previous findings from knock-out models (Mathes et al., 2010).

One of the most prolific lines used in studies of exercise comes from researchers at The University of Michigan. Now in its 35th generation, the rats bred for high capacity running (HCR) and low capacity running (LCR) have been involved in numerous studies. Numerous descriptive studies show divergent physiology between these two lines. LCR rats have altered lipid metabolism (Spargo, 2007), higher risk for cardiovascular disease (Wisløff et al., 2005), and whole-body insulin resistance (Lessard et al., 2009); conversely HCR rats have higher pain thresholds (Geisser et al., 2008), reduced sensitivity to ventricular arrhythmia (Lujan, Britton, Koch & DiCarlo, 2006), and improved oxygen transport and utilization (Gonzalez et al., 2006a).

These traits are responsible for the intrinsic difference in aerobic capacity between these two lines, measured both by longer time to exhaustion on a treadmill, running speed and maximal oxygen uptake (Hoydal, Wisloff, Kemi & Ellingsen, 2001; Koch & Britton et al., 2001). Recently, adaptive differences in galanin expression in the LC were discovered between the HCR and LCR strains, reflecting the benefits of exercise on neural plasticity and function (Murray et al., 2010). Descriptive physiological studies have given way to investigations of the gene-environment interactions possibly responsible for obesity, physical inactivity and fitness (Koch & Britton, 2008a; Koch & Britton 2008b).

One deterrent from using forced treadmill exercise in rodent studies is the fatigue experienced during sustained bouts of physical activity. A recent study of the HCR/LCR lines shows that an upregulation of DA and 5-HT receptor mRNA in the midbrain and striatum of HCR, but not LCR rats, indicates these monoamines possibly mediate the differences in central fatigue experienced by the two lines (Foley et al., 2006). This reflects findings that candidate genes, DRD2, 5-HT2A and 5-HT2C, active in reward pathway modulation are affected by physical activity (Bray, et al., 2009; Dishman, 2008). Current research provides neither conclusive evidence for the mechanisms responsible for these changes, nor substantial data indicating consistent directional changes as a result of physical activity, especially in striatal ENK and DYN differences. Therefore research should focus on a bi-directional effect of voluntary exercise on changes in gene expression in the VTA and striatum, preferably in a selectively bred line, in order to further examine the genetic component of physical activity that may exist in the motor-limbic pathways of the brain. Based on previous lines of evidence described here, we predict that striatal mRNA expression will be decreased and TH mRNA

expression increased in the VTA of HCR females compared to LCR females, and in running animals compared to sedentary animal (Fig. 2.1)

CHAPTER 3

EXPRESSION OF MESSENGER RNA (mRNA) FOR BRAIN DOPAMINE RECEPTORS AND ENKEPHALIN BEFORE AND AFTER WHEEL RUNNING IN RATS SELECTIVELY BRED FOR HIGH- AND LOW- AEROBIC CAPACITY[1]

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Abstract

We examined whether female rats selectively bred for high intrinsic aerobic capacity (High Capacity Runners, HCR) and low intrinsic aerobic capacity (Low Capacity Runners, LCR) differentially express mRNA in the dopaminergic and opiodergic pathways of the basal ganglia and that 3 weeks of access to activity wheels alters expression in those pathways. Consistent with predicted outcomes, enkephalin (ENK) was expressed at lower levels in the nucleus accumbens septi (NAS) and olfactory tubercle (OT) in HCR females compared to LCR females. D_1 receptor mRNA expression in the dorsal striatum and NAS was decreased in rats with running wheel access compared to those that were sedentary for 3 weeks. Contrary to predictions, tyrosine hydroxylase (TH) and D_2 mRNA expressions were not significantly different between HCR and LCR strains and were not affected by wheel running. The results suggest that female rats selectively bred for intrinsically high aerobic capacity demonstrate a high level of voluntary physical activity that is associated with high dopaminergic tone, consistent with low ENK mRNA expression in the basal ganglia. Increased voluntary wheel running results in downregulation of D_1 transcription in the ventral striatum regardless of intrinsic running capacity.

Key words: activity wheel, *in situ* hybridization, opioids, mesolimbic dopaminergic pathway

Introduction

Family and twin studies indicate that variation in human physical activity levels is heritable (Carlsson, Andersson, Lichtenstein, Michaelsson, & Ahlbom, 2006; Eriksson, Rasmussen, & Tynelius, 2006; Lauderdale, et al., 1997; Maia, Thomis & Buenen, 2002; Perusse, Tremblay, Leblanc, and Bouchard, 1989; 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 (Kelly, et al., 2010; Knab & Lightfoot, 2010; Lightfoot, Turner, Daves, Vordermark, & Kleeberger, 2004; Lightfoot, Turner, Pomp, Kleeberger, & Leamy, 2008; Lightfoot et al., 2010; Swallow, Carter, & Garland, 1998) and provides a model of physical activity that allows experimental investigation of neurobiological mechanisms associated with exercise.

Wheel running appears to be a preferred and evolutionarily salient behavior in rodents (Belke, 2005; Brené et al., 2007; Cosgrove, Hunter & Carroll, 2003; Iversen, 1993; Jónás et al., 2010; Lett, 2000; Scarpace, Matheny & Zhang, 2010; Sherwin, 1998). Numerous lines of knockout mice have been used to manipulate genes that may underlie traits that explain locomotory behavior, including skeletal muscle myostatin (Personius et al., 2010) and dopamine (Doi et al., 2006) and opioid (Kieffer & Gavériaux-Ruff, 2002) receptors. Similarly, rats selectively bred for high or low treadmill performance (high capacity runners (HCR) and low capacity runners (LCR)) (Koch & Britton, 2001) differ widely in their capacity to run on a treadmill to the point of exhaustion (Koch & Britton, 2008) and demonstrate a substantial divergence in running speed, duration, and maximal oxygen uptake (Høydal et al., 2007). These selected line differences are associated with several traits subordinate to exercise performance including a greater capacity of HCR to deliver and utilize O_2 in skeletal muscle (Howlett et al.,

2009). However, these differences do not fully account for the wide differences in running behavior between the HCR and LCR lines and may reflect traits that influence the drive to engage in motor behavior (Jónás et al., 2010; Novak et al., 2010). Here, we use the HCR/LCR model to investigate the effects of intrinsic differences in running behavior on dopamine and opioidergic transcriptional differences in the midbrain and striatum.

Although the neurobiology of motivated wheel running is as yet unknown (Sherwin, 1998), it is plausibly manifest as a result of interactions between multiple signaling pathways in the mesolimbic-motor interface (Burgess, 2010; Knab, Bowen, Hamilton, Gulledge, & Lightfoot, 2009; Scheurink, Boersma, Negardh, and Sodersten, 2010), which evidence suggests exists at the basal ganglia (Garcia-Rill, 1986; Jahn et al., 2004; Marsden & Obeso, 1994; Mogenson, 1987, Parent & Hazrati, 1995; Smith, Feldman & Schmidt, 1988, Takakusaki, Oohinata-Sugimoto, Saitoh & Habaguchi, 2004). Specifically, dopaminergic signaling from the ventral tegmental area (VTA) to distinct areas of the striatum has received significant attention for its role in motivated behavior and its incorporation in the limbic system (Cardinal, Parkinson, Hall & Everitt, 2002; Depue & Collins, 1999; Horvitz, 2002).

Studies have indicated a robust effect of striatal dopaminergic activation on opioid gene expression in the olfactory tubercle (OT) (LeMoine et al., 1990; Young, Bonner & Brann, 1986), an effect that has been implicated in driving both natural reward and addiction (Ikemoto, 2007). Additionally, pharmacological denervation of the midbrain-striatal dopamine (DA) pathway decreases locomotion (Joyce & Koob, 1981; Koob et al., 1978), which is plausibly mediated by altered gene expression for dopamine receptors D_1 and D_2 and subsequent effects on enkephalin (ENK) (Churchill et al., 1998; Holmes, 1999; Primeaux & Holmes, 2000).

Brain dopaminergic activity increases in response to acute (Hattori Naoi & Nishino, 1994) and chronic (Gilliam et al., 1984) treadmill training, as do ENK concentrations in the basal ganglia (Blake et al., 1994). The rate limiting enzyme for DA synthesis, tyrosine hydroxylase (TH), increases with chronic stress and drug administration (Dishman, 1997; Dishman et al., 1997; Lu et al., 2003; Nestler, 1992), but only a few studies have empirically demonstrated that chronic, voluntary exercise increases striatal dopaminergic activity in the VTA (Foley & Fleshner, 2008; Greenwood et al., 2010; Heyes, Garnett, & Coates, 1988). There is also evidence that D_1 gene expression is downregulated in the nucleus accumbens septi (NAS) of highly-active animals compared to less-active animals, although the effect could be independent of TH activity (Knab et al., 2009).

D_1 and D_2 receptors are co-localized on neurons opiodergic neurons in the striatum. D_2 receptors are expressed in high concentrations on ENK neurons, and inhibit activation of striato-pallidal projections to the ventral pallidum creating an indirect pathway to the VTA (Engber et al., 1992; Lu et al., 1997). Increased DA transmission to the striatum, as reflected by decreased D_2 mRNA expression, would increase activity of inhibitory GABA neurons from the ventral pallidum which synapse on the subthalamic nuclei, inhibiting tonically active glutamatergic efferent neurons to the VTA (Groenewegen, 2003). Xu et al. (1994) found that hyperactivity in D_1 receptor deficient mice was reflective of greater intrinsic DA release by the midbrain. This suggests D_1 receptor activation acts to inhibit striatal dopaminergic innervation from the VTA through a direct feedback pathway from the striatum (Yung et al., 1995).

To our knowledge, no study has examined the effect of intrinsic aerobic running capacity and chronic wheel-running exposure on dopamine driven changes in opioid gene expression in

the basal ganglia. Here, we report on rats selectively bred for high or low aerobic running capacity. These lines of rats provide a novel approach for simultaneously investigating differences in intrinsic drive to exercise, and activity-induced adaptations in the mesolimbic dopamine system. We hypothesized that rats having high intrinsic running capacity would have greater TH transcription in the VTA, lesser D_1 and D_2 mRNA expression in the striatum, and therefore lesser striatal ENK mRNA expression than rats with low intrinsic running capacity. Likewise, we hypothesized that 3 weeks of wheel-running would increase TH mRNA expression in the VTA, thereby decreasing dopamine receptor expression at D_1 and D_2 sites in the ventral striatum, leading to downregulation of ENK mRNA in the striatum compared to sedentary animals.

Materials and Methods

Animals and Experimental Design

Adult, female rats of two strains were housed individually in 30x30x30 polycarbonate cages (N=40; n=20 HCR, n=20 LCR) 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 over three weeks during adolescence (Table 3.1). Animals underwent a two-week quarantine and facility adaptation period in group housing. without wheels, prior to random allocation, blocked by strain, to activity wheel (AW) or sedentary (SED) conditions. Allocation was performed using Research Randomizer (*www.randomizer.org*). The voluntary running and sedentary control groups each consisted of 10 HCR and 10 LCR rats. All

procedures were approved by the institutional animal use committee and conducted in accordance with NIH Guide for Care and Use of Laboratory Animals.

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. AW 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.

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 ventral tegmental area (VTA) 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, Chambliss, Holmes, & Dishman, 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 be dehydrated through a series of ethanol washes, delipidated in chloroform, rinsed again in ethanol, and allowed to dry.

Oligonucleotide probes were obtained from Oligos, Etc. (Wilsonville, OR)., were 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) size exclusionary columns were utilized to separate unicorporated nucleotides from 4 of the probes. The columns were discontinued by the vendor, so D_1 was separated using BioRad (Hercules, CA) size exclusionary spin columns. Sections were hybridized with the radiolabeled probes 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. Sections were incubated overnight at 37^{o}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 to autoradiographic film for four weeks.

Image Analysis

Autoradiographic film was photographed using an overhead camera (Nikon D5000) operated through CameraControl on a Macintosh computer, with film placed on a light table. Analysis was conducted, blind to assignment and strain, using ImageJ (version 1.44). Gray-scale was converted to μCi values using a radioactive standard, with a calibration performed for each probe. The areas of interest for each probe were compared to background. Measurements were taken at points bilaterally on each section at 6 sites for all probes: left and right, dorsal striatum, (NAS), and OT, except TH which was taken at 2 sites (left and right VTA). Background was recorded for each slide (Fig. 3.1). Outliers were omitted within each site across all slides using Grubbs Criteria (Grubbs & Beck, 1972), and as a result one (1) HCR female was removed from analysis of ENK expression in the OT.

Attrition from tissue loss was balanced across strain and experimental condition but differed by probe and region. ENK in dorsal striatum: 8AW (4 HCR and 4 LCR), and 5 SED (4

HCR and 1 LCR); ENK in NAS: 8 AW (4 HCR and 4 LCR), and 5 SED (4 HCR and 1 LCR); ENK in OT: 8 AW (4 HCR and 4 LCR), and 5 SED (4 HCR and 1 LCR); D_1 in dorsal striatum: 3 AW (1 HCR and 2 LCR), and 3 SED (2 HCR and 1 LCR); D_1 in NAS: 4 AW (2 HCR and 2 LCR), and 3 SED (2 HCR and 1 LCR); D_1 in OT: 5 AW (2 HCR and 3 LCR), and 5 SED (4 HCR and 1 LCR); D_2 in dorsal striatum: 6 AW (4 HCR and 2 LCR), and 3 SED (1 HCR and 2 LCR); D_2 in NAS: 6 AW (4 HCR and 2 LCR), and 3 SED (1 HCR and 2 LCR); D_2 in OT: 8 AW (5 HCR and 3 LCR), and 4 SED (2 HCR and 2 LCR); TH in VTA: 1 AW (1 LCR), and 1 SED (1 LCR).

There was high agreement (ICC (2, 4), Cronbach α = .819 to .921) across sections and hemispheres for D1, D2, and ENK (Table 3.2) and TH (ICC (2,4), Cronbach α = .842), so the mean of values across hemispheres and sections within each animal was calculated.

Data Analysis

Wheel running distance over time was analyzed with a 2 group (HCR vs. LCR) x 3 time (weeks 1-3) mixed-model repeated measures analysis of variance (RM-ANOVA). The Huynh-Feldt ε correction for sphericity violation was used. Levels (μCi) of mRNA for each variables in each brain region were compared using a 2 group (HCR vs. LCR) x 2 condition (activity wheel vs. sedentary) ANOVA with Bonferroni follow up contrasts. Effect sizes were estimated by η^2. Linear regression analysis was used to assess the effects of daily running distance on mRNA for each probe. The sample size was sufficiently powered to detect moderately large effect sizes ($\eta^2 \geq .15$) at a statistical power >.80, and $p < .05$. All analyses were conducted using SPSS Windows version 18.0 (SPSS, Inc., Chicago, IL).

Results

Running Distance and Body Weight

Weekly running was reliable across the three weeks, ICC (2,3) =.875, and increased over time, $F(2,36)=14.486$, $\varepsilon=.846$, $\eta^2 = .45$, $p<.001$. There was an effect of strain, $F(1, 18)=47.289$, $\eta^2 = .72$, $p<.001$. HCR rats ran more on average than LCR rats, $p<.001$, (Fig. 3.2), but there was also a strain x quadratic trend across time, $F(1,18) = 10.192$, $\eta^2 = .23$, $p=.032$. Weekly running distance increased linearly in LCR $F(1,9) = 12.212$, $\varepsilon=.564$, $\eta^2 = .58$, $p=.007$, but it reached a plateau after week 2 in HCR $F(1,9) = 8.168$, $\varepsilon=.908$, $\eta^2 = .48$, $p=.017$.

Body weight was reliable across the three weeks, ICC (2,3) =.999, and increased linearly over time, $F(2,72)=110.161$, $\varepsilon= 1.0$, $\eta^2 = .75$, $p<.001$. There was an effect of strain, $F(1, 36)=120.185$, $\eta^2 = .77$, $p<.001$. HCR rats weighed less on average (initial mean ± SD; pre-decapitation mean ± SD) (162 ± 11 gm; 203 ± 17 gm) than LCR rats (209 ± 15 gm; 259 ± 16 gm), but time x strain and time x strain x wheel running effects were not significant between AW rats (184 ± 27 gm; 223 ± 33 gm), and SED rats (187 ± 28 gm; 239 ± 31 gm) (F-values < 1.98, $\eta^2 < .06$, *p-values* >.145).

ENK mRNA in the Striatum

There was a strain effect in the NAS, $F(1,23)=4.905$, $\eta^2 = .18$, $p =.037$, and the OT, $F(1,23) =13.302$, $\eta^2 = .37$, $p =.001$ (figures 3.3 and 3.4). HCR expressed less ENK mRNA compared to LCR. A similar effect in the dorsal striatum did not reach statistical significance, $F(1,23) = 3.981$, $\eta^2 = .15$, $p=.058$. Wheel running and strain x wheel running effects were not statistically significant (F-values < 1.555, $\eta^2 < .07$, $p >.221$. ENK mRNA was inversely correlated with average running distance in the NAS, $r = -.631$, , $t = 2.573$, $p = .028$, and the OT,

r = -.740, t = 3.48, p=.008, but those relations were explained by the strain effect of higher running distance and lower ENK mRNA in HCR rats compared to LCR rats. After adjusting for strain, the correlations were no longer significant in the NAS, (β = -.813, t = 1.581, p = .148) and the OT (β = -.216, t = 0.910, p = .387) (Fig 3.5). ENK mRNA was weakly related to running distance in the dorsal striatum (r = -.479, t = 1.724, p = .115), but that relation was also explained by the higher running and lower ENK mRNA in HCR compared to LCR rats.

D_1 mRNA in the Striatum

There was a wheel running effect in the NAS, $F(1,29)=4.687$, $\eta^2 = .14$, p=.039, and the dorsal striatum, $F(1,30)=4.742$, $\eta^2 = .14$, p=.037 (figures 3.6 and 3.7). Runners expressed less D_1 than sedentary animals. Other effects of strain and wheel running were not statistically significant (F-values < 2.386, $\eta^2 < .08$, *p-values* >.132). The strain x wheel running effect in the OT approached signficance (F-values = 3.905, $\eta^2 < .13$, p =.059) (Fig. 3.8). D_1 expression was not significantly correlated with average running distance at any of the 3 sites (r-values = -.131 to .434, p-values > .106).

D_2 mRNA in the Striatum

There were no strain, wheel running, or strain x wheel running effects (F-values < 3.557, $\eta^2 < .13$, *p-values* >.070).

TH mRNA in the VTA

The was no effect of strain, $F(1, 34) = 2.892$, $\eta^2 = .08$, p = .098, or wheel running, $F(1, 34) = .001$, $\eta^2 = .000$, p= .979, and there was no strain x wheel running effect, $F(1, 34) = .146$, η^2=.004, p=.705.

Discussion

We report that rats selectively bred for high aerobic running capacity have intrinsically lower gene expression for ENK in the NAS and the OT and that wheel running down regulates D1 receptor mRNA within the ventral striatum. Transcription of ENK was negatively correlated with running distance, but that relation was explained by lower ENK mRNA and higher running distances that were characteristic of HCR compared to LCR animals. Contrary to expectations, there were no significant strain differences or effects of wheel running on mRNA expression for TH in the VTA or for D_2 in the striatal regions.

Our results suggest that selective breeding for aerobic capacity in rats results in altered gene expression in enkephalinergic neurons of the mesolimbic reward system, and that three-weeks of wheel running induces adaptations in D_1 receptor transcription. Because of the lack of consistent differences in gene expression across all probes among groups differing in running capacity and allocation to wheel running, a dopamine driven model of striatal opioid changes as an explanation for differences in voluntary physical activity between HCR and LCR females is suggested, but not fully supported.

The findings are only partly consistent with our hypothesis that opioidergic transcriptional changes in the striatum are mediated by exercise induced midbrain dopamine transmission to the striatum. An early study showed increased DA activity in the striatum during acute exercise bouts on a treadmill (Hattori, Naoi & Nishino, 1994). Additionally, effects of endurance treadmill training were found on striatal DA metabolism and D_2 receptor function (MacRae, Spirduso, Cartee, Farrar & Wilcox, 1987; MacRae, Spirduso, Walters, Farrar & Wilcox, 1987). While these findings suggest that a dopamine-driven mechanism might regulate

physical activity, the forced nature of treadmill running precludes direct comparison to activity wheel running (Dunn, Reigle, Youngstedt, Armstrong & Dishman, 1996; Moraska, Deak, Spencer, Roth & Fleshner, 2000).

TH mRNA expression is one way to measure dopaminergic changes, but it is possible that receptor characteristics not measured are responsible for observed changes in neuropeptide and receptor expression. It is plausible that differences in midbrain DA activity, while not reflected in TH mRNA expression, could alternatively be measured indirectly by expression of the dopamine transporter gene (*DAT1*) (Rhodes et al., 2001). Such differences would operate to inhibit antagonistic feedback to midbrain DA output from the VTA (Steiner & Gerfen, 1998). HCR rats may have greater basal quantities of TH available in the VTA, and therefore do not require transcriptional changes in TH to produce more dopamine. The seminal study indicating wheel-running induced upregulation in TH employed a protocol lasting six weeks, with intermittent conditioning training (Greenwood et al., 2010). As DA decreases across the animal's lifespan, it is possible that the perceived TH upregulation in animals with access to running wheels is actually an age mediated decrease in DA in the control animals and subsequent downregulation of TH in response to sedentary conditions (Dickerson et al., 2009). Our use of adolescent rats and 3-weeks of random assignment rules out these effects.

Our data suggest that intrinsic drive to exercise is marked by lower ENK mRNA expression, which suggests increased DA release from the VTA in HCR female rats compared to LCR female rats. These alterations in ENK neurons are not affected chronically by exposure to either sedentary housing or wheel running. No consensus exists on the effects of physical activity on ENK mRNA expression, but our lack of significant findings mirror those by

Bjornebekk, Mathé, & Brené (2005). Of two human studies, one suggests an acute temporal effect of exercise on plasma met-enkephalin levels (Boone, Sherraden, Pierzchala, Berger & Van Loon, 1992), while another found the effect to be intensity dependent (Sommers et al., 1990), though an early study demonstrated that plasma ENK levels cannot be considered to reflect those in the CNS due to the semipermeable nature of the blood-brain barrier (Cornford, Braun, Crane & Oldendorf, 1978). Chronic effects of striatal denervation, through lesions of TH containing cells, yielded a two-week increase in ENK mRNA, suggesting that DA activation of the D_2 receptor inhibits ENK release, and in agreement with previous findings that D_2 receptors inhibit release of protein kinases necessary for phosphorylation of transcription factors (Gerfen, et al., 1990; Li et al., 1990; Young, Bonner & Brann, 1986). Foley and colleagues (2006) also demonstrated that HCR animals had intrinsically greater D_2 receptor concentrations which could reduce ENK transcription. Coupled with our findings, this evidence suggests that HCR animals may have greater dopaminergic tone between the midbrain and striatum, compared to LCR. Therefore, motivation to exercise that occurs in those with greater aerobic capacity, might be driven by increased tonic midbrain DA release which in turn decreases ENK expression. ENK-mediated indirect inhibitory control of the globus pallidus might therefore increase, resulting in net motor cortex activation.

Without data indicating chronic changes in D_2 receptor transcription to physical activity, it is unlikely to expect running-induced changes in ENK. It is possible then, that effects of physical activity are limited to the direct pathway, as reflected in transcriptional changes in D_1, and D_2 and ENK, in the indirect pathway from the striatum, may be responsible for tonic control of the VTA and motor cortex.

We are unaware of evidence supporting a mechanism of non-equivalent D_1 and D_2 receptor mRNA regulation as a result of chronic wheel running, but we recognize some comparisons were insufficiently powered after animal attrition. There is also limited evidence that D_1 receptors exist in some capacity in ENK-expressing neurons in the striatum, though the *in situ* technique doesn't allow for specificity necessary to identify co-localization. Nonetheless, our results mirror conclusions drawn by Rhodes & Garland (2003) that increased wheel running in mice selectively bred for hyperactivity is mitigated by D_1, but not D_2, differences compared to a non-selected, control line. Similarly, Knab et al. (2009) failed to find training effects on gene expression in the dopamine system using male C57L/J (high active) and C3H/HeJ (low active) mice, but found a strain difference in the D_1 receptor gene. Decreased D_1 receptor mRNA expression in the NAS and the OT in animals exposed to activity wheels, compared to sedentary counterparts, lends support to the theory that DA is dependently linked to voluntary wheel running (Knab & Lightfoot, 2010). Increased chronic binding at D_1 receptors could reduce inhibitory feedback to the VTA, thereby promoting further DA release. Additionally, decreases in D_1 receptor mRNA found in the dorsal striatum are consistent with findings by Hersch et al. (1995) that D_1 receptors localized here, while less concentrated than D_2 receptors, have greater synaptic activation by ipsilateral neuronal afferents linking the striatum and motor cortex.

We propose that running alters basal ganglia function, and that the effects are mediated by midbrain D_1 receptor changes. Furthermore, our results suggest intrinsic differences in aerobic running capacity may be driven by transcriptional differences in striatal enkephalanergic neurons. Our use of rats selectively bred for aerobic capacity and targeting of specific candidate genes contrasts previous literature (Leamy, Pomp & Lightfoot, 2010; Lightfoot et al., 2007;

Lightfoot et al., 2010), while our finding that ENK possibly acts independently to mediate physical activity fills a void left by past research (Knab & Lightfoot, 2010). The intrinsic differences in ENK mRNA and activity wheel running exhibited by the HCR and LCR females studied here suggest a central mechanism underlying voluntary running behavior, consistent with previous findings of a DA mediated arousal state (Sakuma, 2008; Sarbadhikari & Saha, 2006). It is plausible that high-running animals maintain high levels of chronic physical activity as a result of increased midbrain DA flow to the striatum, and low-running animals do not partake in high levels due to greater tonic DA release from the VTA. Future research using these lines of selectively bred rats should further elucidate the actions of striatal opioids and dopamine receptors as they relate to voluntary physical activity, and in light of recent evidence, expand the scope of basal ganglia influence to include the ventral pallidum (i.e., Borer, 2010; Smith, Berridge, and Aldridge, 2011). Collectively, our findings partly support the hypothesis that individual differences in voluntary physical activity are driven by a gene-environment interaction involving the midbrain dopamine pathways and the limbic-motor junction (Dishman, 2008).

References

Blake, M. J., Stein, E. A., & Vomachka, A. J. (1984). Effects of exercise training on brain opioid peptides and serum LH in female rats. *Peptides, 5*(5), 953-958. doi: 10.1016/0196-9781(84)90122-0

Boone, J. B., Sherraden, T., Pierzchala, K., Berger, R., & Van Loon, G. R. (1992). Plasma Met-enkephalin and catecholamine responses to intense exercise in humans. *Journal of Applied Physiology, 73*(1), 388-392.

Borer, K. T. (2010). Nonhomeostatic control of human appetite and physical activity in

Regulation of Energy Balance. *Exercise and Sport Sciences Reviews, 38*(3), 114-121 110.1097/JES.1090b1013e3181e3728f.

Brené, S., Bjørnebekk, A., Åberg, E., Mathé, A. A., Olson, L., & Werme, M. (2007). Running is rewarding and antidepressive. *Physiology & Behavior, 92*(1-2), 136-140. doi: 10.1016/j.physbeh.2007.05.015

Burgess, C. R. (2010). Histamine and orexin in the control of arousal, locomotion, and motivation. *The Journal of neuroscience, 30*(8), 2810-2811.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience & Biobehavioral Reviews, 26*(3), 321-352. doi: 10.1016/s0149-7634(02)00007-6

Carlsson, S., Andersson, T., Lichtenstein, P., Michaelsson, K., & Ahlbom, A. (2006). Genetic effects on physical activity: Results from the Swedish Twin Registry. *Medicine & Science in Sports & Exercise, 38*(8), 1396-1401 1310.1249/1301.mss.0000228941.0000217034.c0000228941.

Churchill, L., Klitenick, M. A., & Kalivas, P. W. (1998). Dopamine depletion reorganizes projections from the nucleus accumbens and ventral pallidum that mediate opioid-induced motor activity. *J Neurosci, 18*(19), 8074-8085.

Cornford, E. M., Braun, L. D., Crane, P. D., & Oldendorf, W. H. (1978). Blood-brain barrier restriction of peptides and the low uptake of enkephalins. *Endocrinology, 103*(4), 1297-1303.

Cosgrove, K. P., Hunter, R. G., & Carroll, M. E. (2002). Wheel-running attenuates intravenous cocaine self-administration in rats: Sex differences. *Pharmacology Biochemistry and*

Behavior, 73(3), 663-671. doi: 10.1016/s0091-3057(02)00853-5

Curran, E. J., & Watson, S. J. (1995). Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: A double in situ hybridization study. *The Journal of Comparative Neurology, 361*(1), 57-76. doi: 10.1002/cne.903610106

Depue, R. A., & Collins, P. F. (1999). Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. *Behav Brain Sci, 22*(3), 491-517; discussion 518-469.

Dickerson, J. W., Hemmerle, A. M., Numan, S., Lundgren, K. H., & Seroogy, K. B. (2009). Decreased expression of ErbB4 and tyrosine hydroxylase mRNA and protein in the ventral midbrain of aged rats. *Neuroscience, 163*(1), 482-489. doi: 10.1016/j.neuroscience.2009.06.008

Dishman, R. K. (1997). Brain monoamines, exercise, and behavioral stress: Animal models. *Medicine and science in sports and exercise, 29*(1), 63.

Dishman, R. K. (2008). Gene-physical activity interactions in the etiology of obesity: Behavioral considerations. *Obesity, 16*(Supplement 3s), S60-S65.

Dishman, R. K., Renner, K. J., Youngstedt, S. D., Reigle, T. G., Bunnell, B. N., Burke, K. A., et al. (1997). Activity wheel running reduces escape latency and alters brain monoamine levels after footshock. *Brain Research Bulletin, 42*(5), 399.

Doi, M., Yujnovsky, I., Hirayama, J., Malerba, M., Tirotta, E., Sassone-Corsi, P., & Borrelli, E. (2006). Impaired light masking in dopamine D2 receptor-null mice. [10.1038/nn1711]. *Nat Neurosci, 9*(6), 732-734. doi: http://www.nature.com/neuro/journal/v9/n6/suppinfo/nn1711_S1.html

Dunn, A. L., Reigle, T. G., Youngstedt, S. D., Armstrong, R. B., & Dishman, R. K. (1996). Brain norepinephrine and metabolites after treadmill training and wheel running in rats. *Medicine & Science in Sports & Exercise, 28*(2), 204-209.

Engber, T. M. (1992). Dopaminergic modulation of striatal neuropeptides: differential effects of D1 and D2 receptor stimulation on somatostatin, neuropeptide Y, neurotensin, dynorphin and enkephalin. *Brain Research, 581*(2), 261.

Eriksson, M., Rasmussen, F., & Tynelius, P. (2006). Genetic factors in physical activity and the equal environment assumption – the Swedish Young Male Twins Study. *Behavior Genetics, 36*(2), 238-247.

Foley, T., & Fleshner, M. (2008). Neuroplasticity of dopamine circuits after exercise: Implications for central fatigue. *NeuroMolecular Medicine, 10*(2), 67-80.

Garcia-Rill, E. (1986). The basal ganglia and the locomotor regions. *Brain Research Reviews, 11*(1), 47-63. doi: 10.1016/0165-0173(86)90009-3

Garcia-Rill, E., Skinner, R. D., & Fitzgerald, J. A. (1985). Chemical activation of the mesecephalic locomotor region. *Brain research, 330*(1), 43-54. doi: 10.1016/0006-8993(85)90006-x

Gerfen, C., Engber, T., Mahan, L., Susel, Z., Chase, T., Monsma, F., et al. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science, 250*(4986), 1429-1432.

Gilliam, P. E., Spirduso, W. W., Martin, T. P., Walters, T. J., Wilcox, R. E., & Farrar, R. P. (1984). The effects of exercise training on [3H]-spiperone binding in rat striatum. *Pharmacology Biochemistry and Behavior, 20*(6), 863-867. doi: 10.1016/0091-

3057(84)90008-x

Graybiel, A. M. (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends in Neurosciences, 13*(7), 244-254. doi: 10.1016/0166-2236(90)90104-i

Greenwood, B. N., Foley, T. E., Le, T. V., Strong, P. V., Loughridge, A. B., Day, H. E. W., et al. (2010). Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behavioural Brain Research, 217*(2), 354-362.

Groenewegen, H. J. (2003). The basal ganglia and motor control. *Neural Plast, 10*(1-2), 107-120.

Grubbs, F. E. & Beck, G. (1972). Extension of sample sizes and percentage points for significance tests of outlying observations. *Technometrics, 14*(4), 847.

Hanson, R. W., & Hakimi, P. (2008). Born to run; the story of the PEPCK-Cmus mouse. *Biochimie, 90*(6), 838-842. doi: 10.1016/j.biochi.2008.03.009

Hattori, S., Naoi, M., & Nishino, H. (1994). Striatal dopamine turnover during treadmill running in the rat: Relation to the speed of running. *Brain Research Bulletin, 35*(1), 41-49.

Herrero, M.-T., Barcia, C., & Navarro, J. (2002). Functional anatomy of thalamus and basal ganglia. *Child's Nervous System, 18*(8), 386-404. doi: 10.1007/s00381-002-0604-1

Hersch, S. M., Ciliax, B. J., Gutekunst, C. A., Rees, H. D., Heilman, C. J., Yung, K. K., . . . Levey, A. I. (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J Neurosci, 15*(7 Pt 2), 5222-5237.

Heyes, M. P., Garnett, E. S., & Coates, G. (1988). Nigrostriatal dopaminergic activity is increased during exhaustive exercise stress in rats. *Life Sciences, 42*(16), 1537-1542. doi: 10.1016/0024-3205(88)90011-2

Holmes, P. V. (1999). Olfactory bulbectomy increases prepro-enkephalin mRNA levels in the ventral striatum in rats. *Neuropeptides, 33*(3), 206-211.

Horvitz, J. C. (2002). Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. *Behavioural Brain Research, 137*(1-2), 65-74. doi: 10.1016/s0166-4328(02)00285-1

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain research reviews, 56*(1), 27.

Ikemoto, S., Glazier, B. S., Murphy, J. M., & McBride, W. J. (1997). Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *J Neurosci, 17*(21), 8580-8587.

Jahn, K., Deutschländer, A., Stephan, T., Strupp, M., Wiesmann, M., & Brandt, T. (2004). Brain activation patterns during imagined stance and locomotion in functional magnetic resonance imaging. *NeuroImage, 22*(4), 1722-1731.

Jónás, I., Schubert, K., Reijne, A., Scholte, J., Garland, T., Gerkema, M., . . . van Dijk, G. (2010). Behavioral traits are affected by selective breeding for increased wheel-running behavior in mice. *Behavior Genetics, 40*(4), 542-550. doi: 10.1007/s10519-010-9359-8

Joyce, E. M., & Koob, G. F. (1981). Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. *Psychopharmacology, 73*(4), 311-313.

Kelly, S. A., Nehrenberg, D. L., Peirce, J. L., Hua, K., Steffy, B. M., Wiltshire, T., et al. (2010). Genetic architecture of voluntary exercise in an advanced intercross line of mice. *Physiol.*

Genomics, physiolgenomics.00028.02010.

Kieffer, B. L., & Gavériaux-Ruff, C. (2002). Exploring the opioid system by gene knockout. *Progress in Neurobiology, 66*(5), 285-306. doi: 10.1016/s0301-0082(02)00008-4

Knab, A. M., & Lightfoot, J. T. (2010). Does the difference between physically active and couch potato lie in the dopamine system? *Int J Biol Sci, 6*(2), 133-150.

Knab, A. M., Bowen, R. S., Hamilton, A. T., Gulledge, A. A., & Lightfoot, J. T. (2009). Altered dopaminergic profiles: Implications for the regulation of voluntary physical activity. *Behavioural Brain Research, 204*(1), 147-152.

Koob, G. F., Riley, S. J., Smith, S. C., & Robbins, T. W. (1978). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. *Journal of Comparative and Physiological Psychology, 92*(5), 917-927.

Lauderdale, D. S., Fabsitz, R., Meyer, J. M., Sholinsky, P., Ramakrishnan, V., & Goldberg, J. (1997). Familial determinants of moderate and intense physical activity: a twin study. *Medicine & Science in Sports & Exercise, 29*(8), 1062-1068.

Le Moine, C. (1990). Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proceedings of the National Academy of Sciences of the United States of America, 87*(1), 230.

Leamy, L., Pomp, D., & Lightfoot, J. (2010). A search for quantitative trait loci controlling within-individual variation of physical activity traits in mice. *BMC Genetics, 11*(1), 83.

Lenard, N. R., & Berthoud, H.-R. (2008). Central and peripheral regulation of food intake and physical activity: Pathways and genes. *Obesity, 16*(Supplement 3s), S11-S22.

Li, S. J., Jiang, H. K., Stachowiak, M. S., Hudson, P. M., Owyang, V., Nanry, K., et al. (1990). Influence of nigrostriatal dopaminergic tone on the biosynthesis of dynorphin and enkephalin in rat striatum. *Molecular Brain Research, 8*(3), 219-225.

Lightfoot, J. T., Leamy, L., Pomp, D., Turner, M. J., Fodor, A. A., Knab, A., . . . Hamilton, A. (2010). Strain screen and haplotype association mapping of wheel running in inbred mouse strains. *Journal of Applied Physiology, 109*(3), 623-634. doi: 10.1152/japplphysiol.00525.2010

Lightfoot, J. T., Turner, M. J., Knab, A. K., Jedlicka, A. E., Oshimura, T., Marzec, J., . . . Kleeberger, S. R. (2007). Quantitative trait loci associated with maximal exercise endurance in mice. *Journal of Applied Physiology, 103*(1), 105-110. doi: 10.1152/japplphysiol.01328.2006

Lightfoot, J. T., Turner, M. J., Pomp, D., Kleeberger, S. R., & Leamy, L. J. (2008). Quantitative trait loci for physical activity traits in mice. *Physiol. Genomics, 32*(3), 401-408.

Lu, L., Grimm, J. W., Shaham, Y., & Hope, B. T. (2003). Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. *Journal of Neurochemistry, 85*(6), 1604-1613.

Lu, X. Y., Behnam Ghasemzadeh, M., & Kalivas, P. W. (1997). Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience, 82*(3), 767-780. doi: Doi: 10.1016/s0306-4522(97)00327-8

MacRae, P. G., Spirduso, W. W., Cartee, G. D., Farrar, R. P., & Wilcox, R. E. (1987). Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine

metabolite levels. *Neuroscience Letters, 79*(1-2), 138-144.

MacRae, P. G., Spirduso, W. W., Walters, T. J., Farrar, R. P., & Wilcox, R. E. (1987). Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolites in presenescent older rats. *Psychopharmacology, 92*(2), 236-240.

Maia, J. A. R., Thomis, M., & Beunen, G. (2002). Genetic factors in physical activity levels: A twin study. *American journal of preventive medicine, 23*(2), 87-91.

Marsden, C. D., & Obeso, J. A. (1994). The functions of the basal ganglia and the paradox of stereotaxic surgery in Parkinson's disease. *Brain, 117 (Pt 4)*, 877-897.

Mogenson, G.J. (1987) Limbic-motor integration. *Progress in Psychobiology and Physiological Psychology, 12,* 117-170.

Moraska, A., Deak, T., Spencer, R. L., Roth, D., & Fleshner, M. (2000). Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 279*(4), R1321-R1329.

Nestler, E. J. (1992). Molecular mechanisms of drug addiction. *The Journal of neuroscience, 12*(7), 2439-2450.

Novak C.M., Escande C., Burghardt P.R., Zhang M., Barbosa M.T., Chini E.N., Britton S.L., Koch L.G., Akil H., Levine J.A., 2010. Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity. Hormones and Behavior. 58(3), 355-367.

Parent, A., & Hazrati, L.-N. (1995). Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Research Reviews, 20*(1), 91-127. doi:

10.1016/0165-0173(94)00007-c

Personius, K. E., Jayaram, A., Krull, D., Brown, R., Xu, T., Han, B., . . . Welle, S. (2010). Grip force, EDL contractile properties, and voluntary wheel running after postdevelopmental myostatin depletion in mice. *Journal of Applied Physiology, 109*(3), 886-894. doi: 10.1152/japplphysiol.00300.2010

Perusse, L., Tremblay, A., Leblanc, C., & Bouchard, C. (1989). Genetic and environmental influences on level of habitual physical activity and exercise participation. *Am J Epidemiol, 129*(5), 1012-1022.

Primeaux, S. D., & Holmes, P. V. (2000). Olfactory bulbectomy increases met-enkephalin- and neuropeptide-Y-like immunoreactivity in rat limbic structures. *Pharmacology Biochemistry and Behavior, 67*(2), 331-337.

Rhodes, J. S., & Garland, T. (2003). Differential sensitivity to acute administration of Ritalin, apormorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology, 167*(3), 242-250. doi: 10.1007/s00213-003-1399-9

Rhodes, J. S., Hosack, G. R., Girard, I., Kelley, A. E., Mitchell, G. S., & Garland, T., Jr. (2001). Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology (Berl), 158*(2), 120-131. doi: 10.1007/s002130100857

Sakuma, Y. (2008). Neural substrates for sexual preference and motivation in the female and male rat. *Annals of the New York Academy of Sciences, 1129*(1), 55-60. doi: 10.1196/annals.1417.009

Sarbadhikari, S. N., & Saha, A. K. (2006). Moderate exercise and chronic stress produce counteractive effects on different areas of the brain by acting through various neurotransmitter receptor subtypes: a hypothesis. *Theor Biol Med Model, 3*, 33. doi: 10.1186/1742-4682-3-33

Scarpace, P. J., Matheny, M., & Zhang, Y. (2010). Wheel running eliminates high-fat preference and enhances leptin signaling in the ventral tegmental area. *Physiology & Behavior, 100*(2), 173-179. doi: 10.1016/j.physbeh.2010.02.017

Scheurink, A. J. W., Boersma, G. J., Nergårdh, R., & Södersten, P. (2010). Neurobiology of hyperactivity and reward: Agreeable restlessness in Anorexia Nervosa. *Physiology & Behavior, 100*(5), 490-495.

Sherwin, C. M. (1998). Voluntary wheel running: a review and novel interpretation. *Animal Behaviour, 56*(1), 11-27. doi: 10.1006/anbe.1998.0836

Simonen, R. L., Rankinen, T., Perusse, L., Rice, T., Rao, D. C., Chagnon, Y., et al. (2003). Genome-wide linkage scan for physical activity levels in the Quebec Family Study. *Medicine & Science in Sports & Exercise, 35*(8), 1355-1359.

Smith, J., Feldman, J., & Schmidt, B. (1988). Neural mechanisms generating locomotion studied in mammalian brain stem- spinal cord in vitro. *The FASEB Journal, 2*(7), 2283-2288.

Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.1101920108

Sommers, K., Loots, J. M., Simpson, S. F., Meyer, E. C., Dettweiler, A., & Human, J. l. R. (1990). Circulating met-enkephalin in trained athletes during rest, exhaustive treadmill

exercise and marathon running. *European Journal of Clinical Pharmacology, 38*(4), 391-392.

Steiner, H., & Gerfen, C. R. (1998). Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. *Experimental Brain Research, 123*(1), 60-76.

Stubbe, J. H., Boomsma, D. I., Vink, J. M., Cornes, B. K., Martin, N. G., Skytthe, A., et al. (2006). Genetic influences on exercise participation in 37,051 twin pairs from seven countries. *PLoS ONE, 1*(1), e22.

Swallow, J. G., Carter, P. A., & Garland, T. (1998). Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics, 28*(3), 227-237.

Takakusaki, K., Oohinata-Sugimoto, J., Saitoh, K., & Habaguchi, T. (2004). Role of basal ganglia-brainstem systems in the control of postural muscle tone and locomotion. In D. G. S. Shigemi Mori & W. Mario (Eds.), *Progress in Brain Research* (Vol. Volume 143, pp. 231-237): Elsevier.

Van Hoomissen, J. D., Chambliss, H. O., Holmes, P. V., & Dishman, R. K. (2003). Effects of chronic exercise and imipramine on mRNA for BDNF after olfactory bulbectomy in rat. *Brain Research, 974*(1-2), 228-235.

Werme, M., Lindholm, S., Thorén, P., Franck, J., & Brené, S. (2002). Running increases ethanol preference. *Behavioural Brain Research, 133*(2), 301-308.

Werme, M., Thorén, P., Olson, L., & Brené, S. (2000). Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. *European Journal of Neuroscience, 12*(8), 2967-2974.

Xu, M., Moratalla, R., Gold, L. H., Hiroi, N., Koob, G. F., Graybiel, A. M., et al. (1994).

Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. *Cell, 79*(4), 729-742.

Young, W. S., Bonner, T. I., & Brann, M. R. (1986). Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. *Proceedings of the National Academy of Sciences, 83*(24), 9827-9831.

Yung, K.K.L., Bolam, J. P., Smith, A.D., Hersch, S. M., Ciliax, B.J. & Levey, A.I. (1995) Immunocytochemical localization of D1 and D2 dopamine receptors in the rat: Light and electron microscopy. *Neuroscience, 65*(3), 709-730.

Table 3.1 Strain differences in treadmill running performance (Mean ± SEM). High aerobic capacity (HCR) female rats ran farther, faster, and for more time than low aerobic capacity (LCR) females as measured over three treadmill testing sessions at the University of Michigan.

	Best speed (meters/min)	Best distance (meters)	Best time (min)
HCR	45.95±.6	2029.702±55.9	72.95±1.2
LCR (n=20)	20.40±.2	323.428±9.1	21.63±.5

Table 3.2 ICC (2,4) of mRNA expression measured across hemispheres is given for each probe in regions of the striatum. Uniform high correlations indicate that expression was bilaterally reliable, and the averaging of values to conduct omnibus tests was justified.

	ENK mRNA	D1 mRNA	D2 mRNA
Dorsal Striatum	.819	.872	.825
NAS	.886	.921	.893
OT	.833	.865	.901

Figure Captions

Fig. 2.1 A schematic diagram of the opioidergic feedback on ventral tegmental area (VTA) dopaminergic neurons. Large solid arrows indicate the direction of mRNA expression differences in high-aerobic capacity (HCR) compared to low-aerobic capacity (LCR) animals, and animals with activity-wheel access (AW) compared to sedentary assignment (SED).

Fig. 3.1 A) An autoradiographic image of a striatal section depicting areas defined as dorsal striatum, nucleus accumbens septi (NAS) and olfactory tubercle (OT), and B) an autoradiographic image of a midbrain section depicting areas measured bilaterally as the VTA.

Fig. 3.2 Wheel-running activity by week for a 3 week period (values are means with uni-directional SEM bars). HCR females ran more than LCR females ($p<.001$), and there was a strain by quadratic effect of time ($p=.024$) and a weight by linear effect of time ($p=.017$).

Fig. 3.3 Enkephalin (ENK) mRNA expression (μCi) in the NAS (values are means ± SEM). HCR females($n= 12$) had lower ENK transcription in the NAS compared to LCR females($n= 15$) ($p=.037$).

Fig. 3.4 ENK mRNA expression (μCi) in the OT (means ± SEM). HCR females ($n= 12$) had lower ENK transcription in the OT compared to LCR females ($n= 15$) ($p<.001$).

Fig. 3.5 ENK mRNA expression as a function of running distance in the NAS and dorsal striatum. The inverse correlation ($p=.008$) is explained by the intrinsic strain difference in both ENK mRNA and running distance.

Fig. 3.6 D_1 mRNA expression (μCi) in the NAS (means ± SEM). AW females ($n= 16$) expressed more D_1 mRNA in the NAS than SED females ($n= 17$) ($p=.039$).

Fig. 3.7 D_1 mRNA expression (μCi) in the dorsal striatum (means ± SEM). AW females

(*n*= 17) expressed more D_1 mRNA in the dorsal striatum than SED females (*n*=17) (*p*=.037).

Fig. 3.8 D_1 mRNA expression (μCi) in the OT (means ± SEM). There was an interaction of strain (*n*=16 LCR; *n*=14 HCR) and condition (*n*=15 AW; *n*=15 SED) when comparing D_1 mRNA expression in the OT (*p*=.059), though it was explained by age differences between groups.

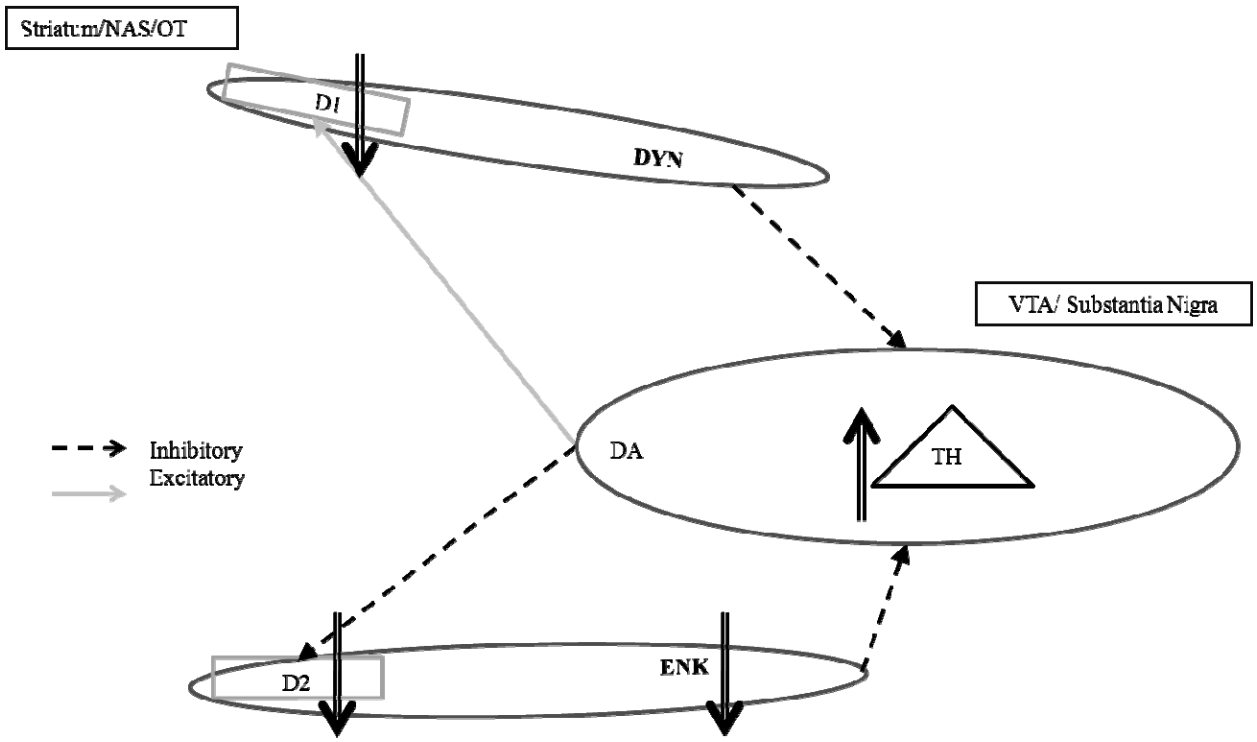
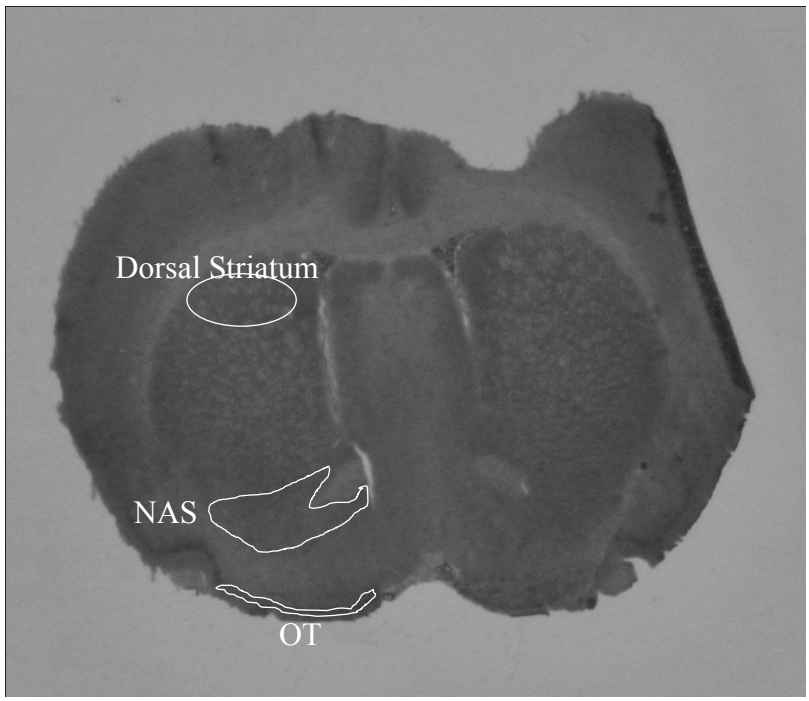
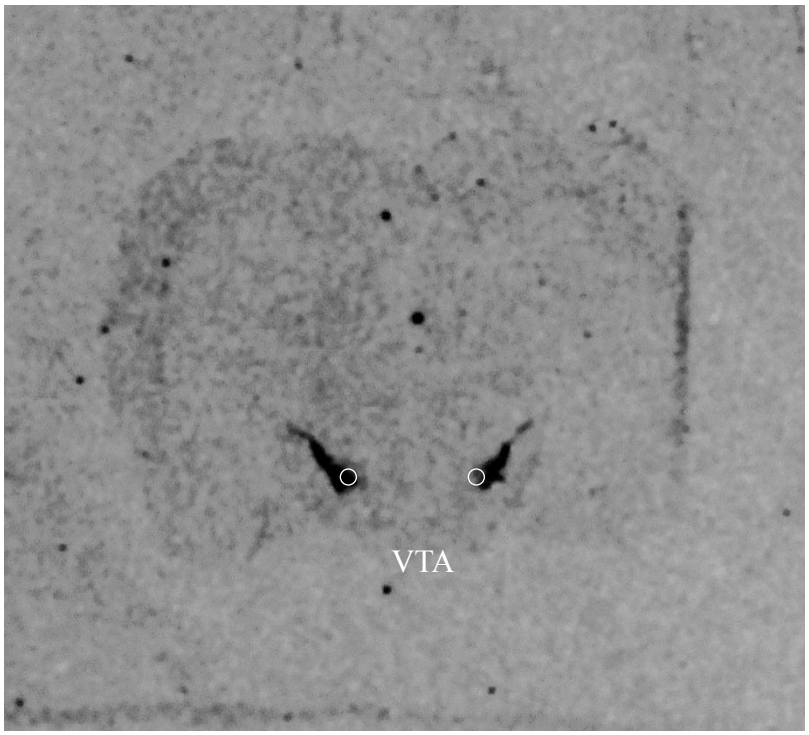

Striatum/NAS/OT
D1
DYN
VTA/ Substantia Nigra
DA
TH
Inhibitory
Excitatory
D2
ENK

Figure 2.1



A)



B)

Figure 3.1

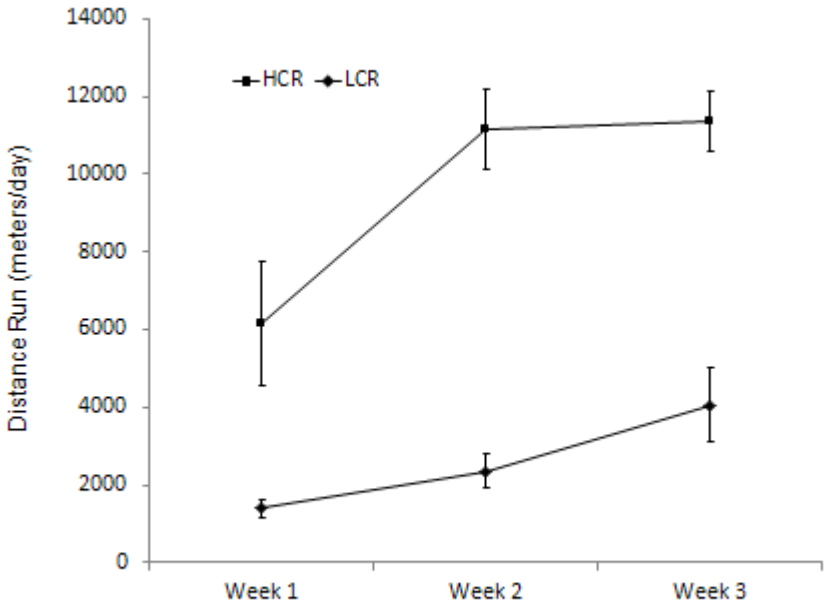

14000
12000
10000
8000
6000
4000
2000
0
Distance Run (meters/day)
HCR
LCR
Week 1
Week 2
Week 3

Figure 3.2

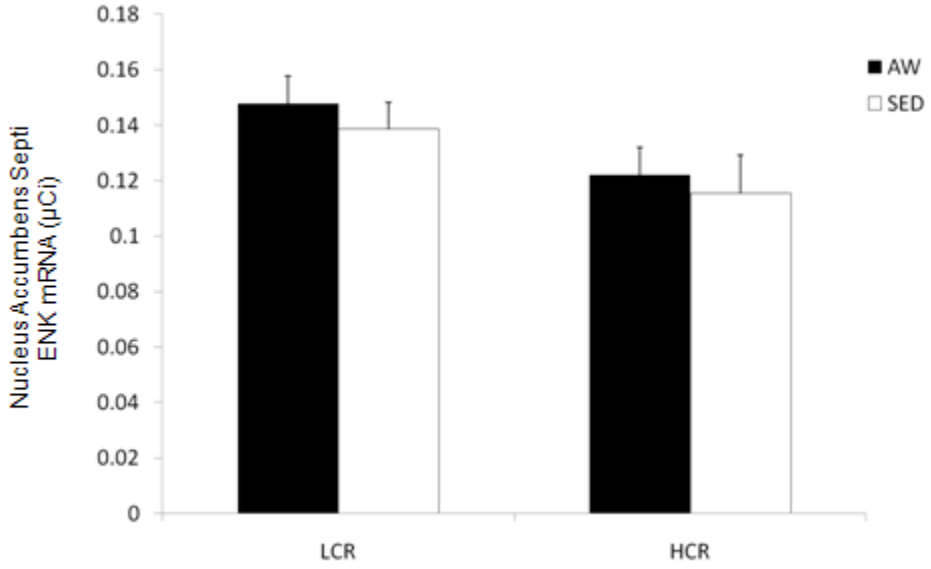


Figure 3.3

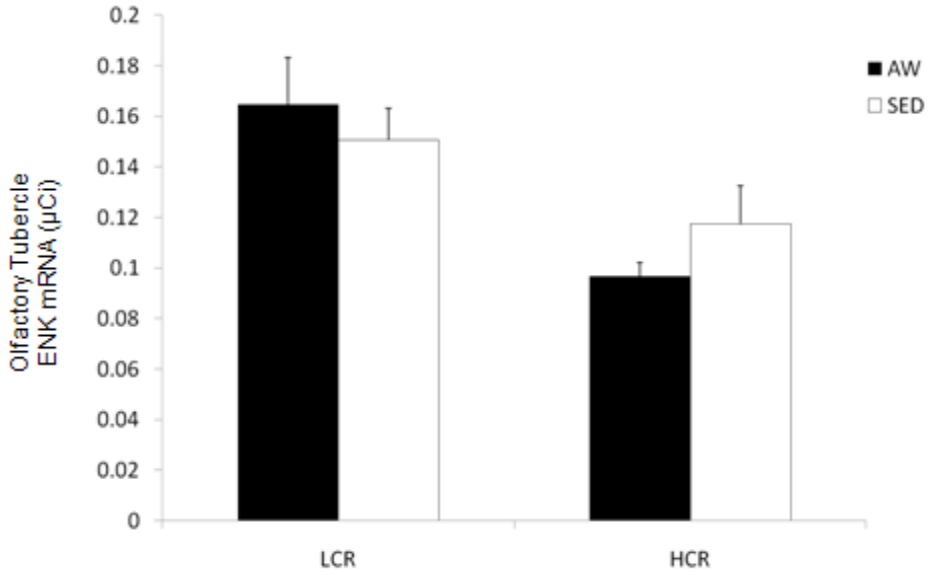
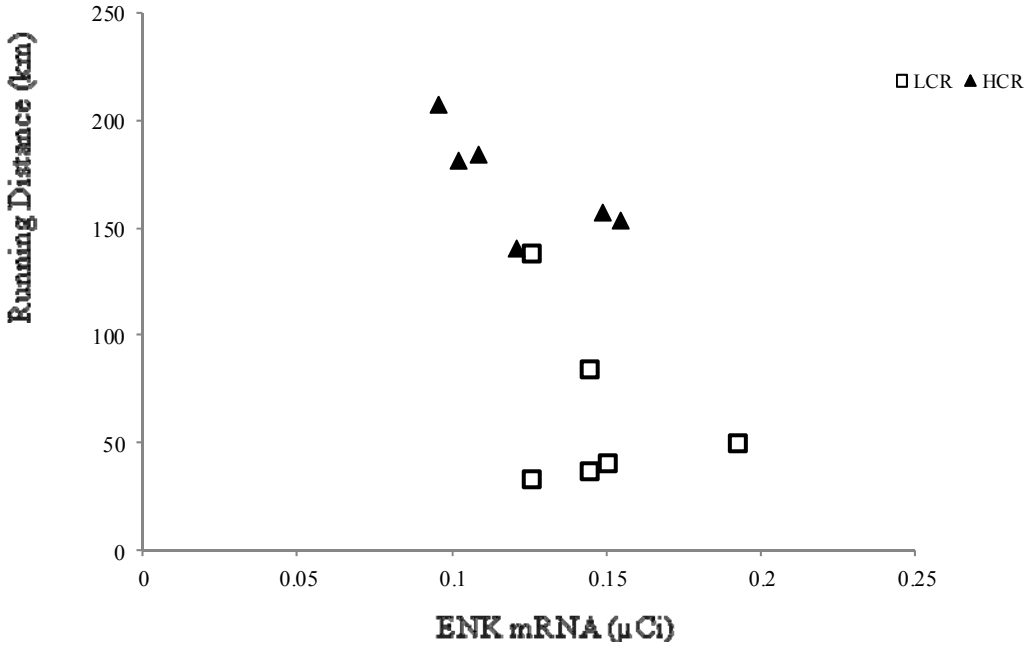


Figure 3.4

NAS



OT

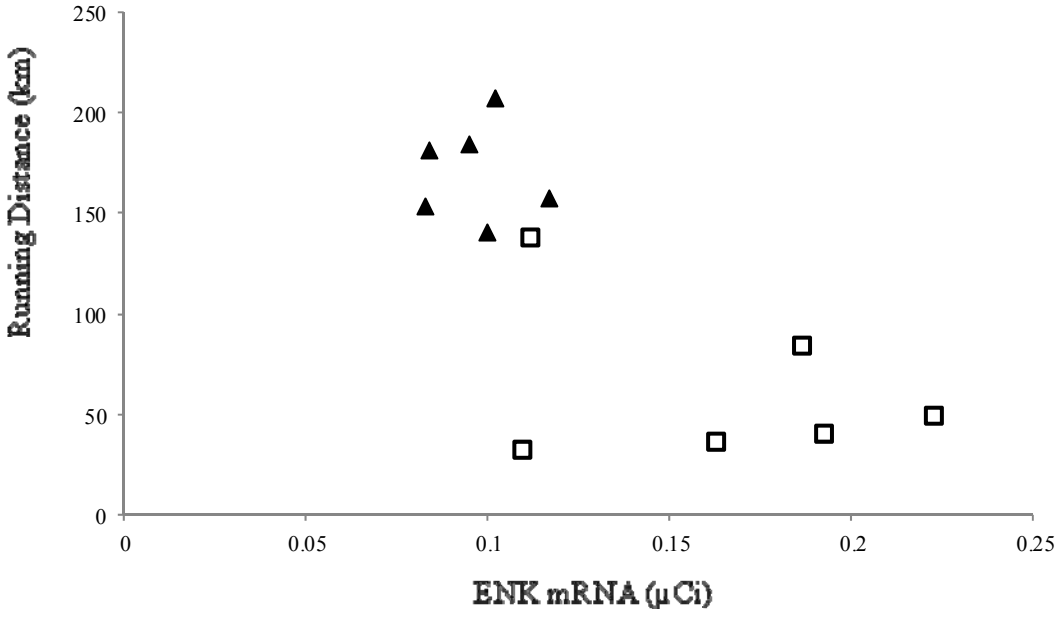


Figure 3.5

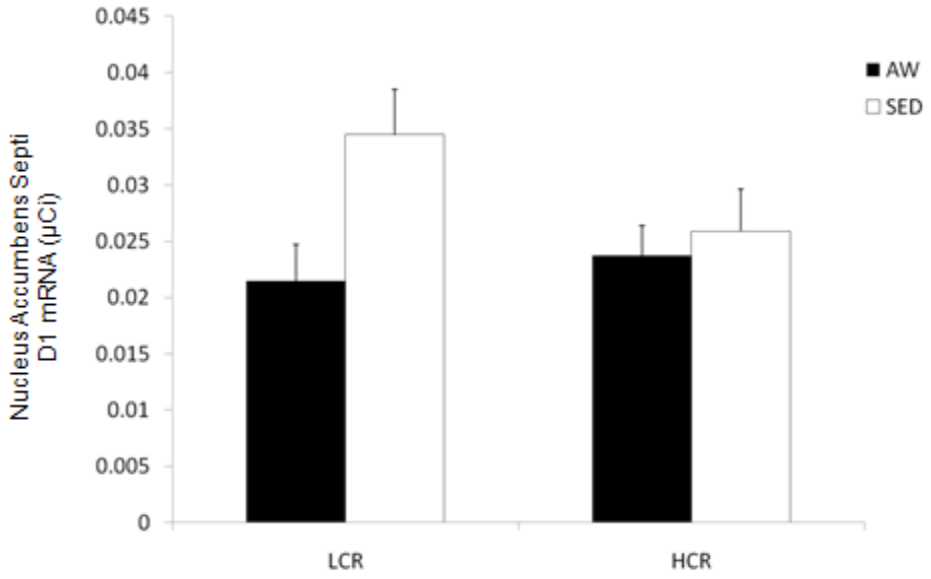


Figure 3.6

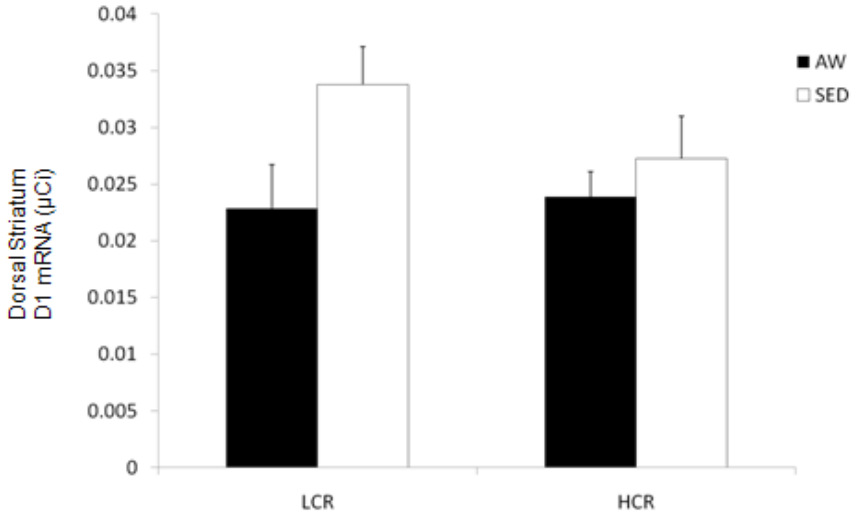


Figure 3.7

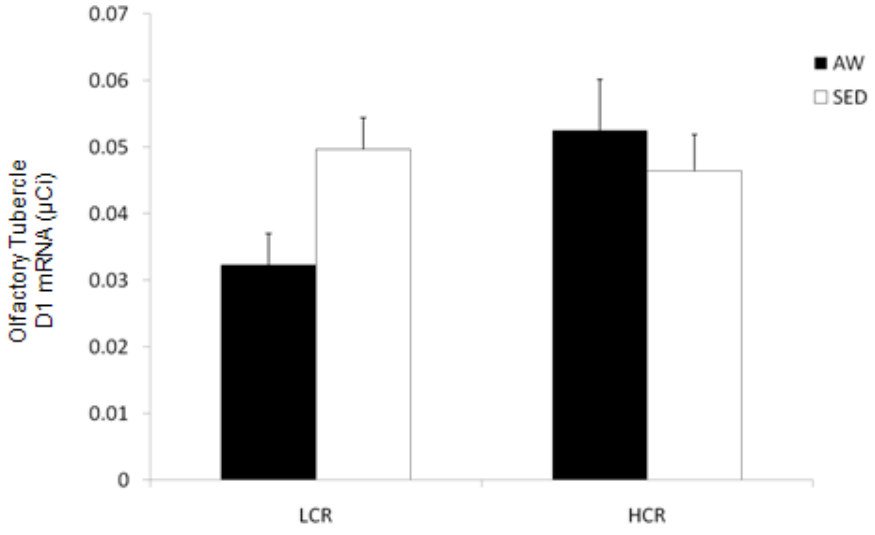

0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Olfactory Tubercle
D1 mRNA (µCi)
AW
SED
LCR
HCR

Figure 3.8

CHAPTER 4

SUMMARY

Our results indicate that ENK mRNA expression in opioidergic pathways known to modulate motivated locomotion is lower in HCR rats compared to LCR rats, and may be partially responsible for divergent running behavior between these two strains. Additionally, D_1 mRNA was upregulated by 3 weeks of access to an activity wheel, suggesting that voluntary wheel running induces adaptive change in the mesolimbic dopaminergic pathways innervating the striatum. Our findings imply that while physical activity may be a heritable trait, motivation to engage in voluntary physical activity has a genetic component that can be altered, at least in part, by activity itself.

Further study of the HCR and LCR line should seek to validate the dopaminergic mechanism that we propose drives changes in this system. Utilizing *in-situ* hybridization changes in DA could also be measured by quantification of the dopamine transporter gene (*DAT1*). Based on our findings, *DAT1* would be expressed more in HCR than LCR rats, and rats with access to an activity wheel would upregulate *DAT1* compared to sedentary animals. There is potential for a dose-response to running, or for an interaction between strain and condition.

Additionally, partially disabling DA neurons in the region using varying administrations of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could serve as a useful manipulation prior to wheel-running. It could be that HCR animals are more resistant to the effects of this neurotoxin.

While numerous confounds accompany rodent training studies due to stress associated with forced-treadmill running, there is potential for training to occur on a wheel. If follow-up studies indicate that HCR rats are indeed more motivated to run on an activity wheel, they may be able to endure greater resistance on the wheel than rodents tested previously. A three part study should be conducted. First, threshold of exertion should be examined in the HCR animals to determine appropriate resistance (as percentages of bodyweight). Second, a graded training program should be administered to these animals, with bodyweight and eating behavior closely monitored to confirm adaptations as a result of this program independent of animal age. Finally, once validity and reliability has been confirmed for training in the HCR strain, studies should commence to describe differences in mesolimbic dopamine pathways and the striatum as a result of training compared to changes in sedentary animals, or those exposed to an activity wheel without resistance. This is an important step in relating findings from rodent studies, to voluntary physical activity as a human behavior.

The idea that physical activity has a genetic component, and that motivation to exercise may at least be partially heritable, is sometimes considered a detriment to the decade-old fight to improve public health. By showing that voluntary physical activity can induce beneficial changes in mRNA expression in areas of the brain associated with learning, reward, and motivation independent of intrinsic differences in levels of physical activity, should instead strengthen the argument for adaptation of a physically-active lifestyle.

REFERENCES

Alex, K. D., & Pehek, E. A. (2007). Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacology & Therapeutics, 113*(2), 296-320.

Balthazar, C. H., Leite, L. H., Ribeiro, R. M., Soares, D. D., & Coimbra, C. C. (2010). Effects of blockade of central dopamine D1 and D2 receptors on thermoregulation, metabolic rate and running performance. *Pharmacol Rep, 62*(1), 54-61.

Barnes, P. (2007). *Physical activity among adults: United States, 2000 and 2005*. Retrieved from http://www.cdc.gov/nchs/data/hestat/physicalactivity/physicalactivity.htm.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain research reviews, 28*(3), 309-369.

Bjornebekk, A., Mathe, A. A., & Brene, S. (2005). The antidepressant effect of running is associated with increased hippocampal cell proliferation. *The International Journal of Neuropsychopharmacology, 8*(03), 357-368.

Bjornebekk, A., Mathe, A. A., & Brene, S. (2005). Running has Differential Effects on NPY, Opiates, and Cell Proliferation in an Animal Model of Depression and Controls. *Neuropsychopharmacology, 31*(2), 256-264.

Blake, M. J., Stein, E. A., & Vomachka, A. J. (1984). Effects of exercise training on brain opioid peptides and serum LH in female rats. *Peptides, 5*(5), 953-958.

Boecker, H., Othman, A., Mueckter, S., Scheef, L., Pensel, M., Daamen, M., et al. (2010). Advocating neuroimaging studies of transmitter release in human physical exercise

challenges studies. *Open Access Journal of Sports Medicine, 1*, 167-175.

Boecker, H., Sprenger, T., Spilker, M. E., Henriksen, G., Koppenhoefer, M., Wagner, K. J., et al. (2008). The runner's high: Opioidergic mechanisms in the human brain. *Cereb. Cortex, 18*(11), 2523-2531.

Boone, J. B., Sherraden, T., Pierzchala, K., Berger, R., & Van Loon, G. R. (1992). Plasma Met-enkephalin and catecholamine responses to intense exercise in humans. *Journal of Applied Physiology, 73*(1), 388-392.

Borer, K. T. (2010). Nonhomeostatic control of human appetite and physical activity in regulation of energy balance. *Exercise and Sport Sciences Reviews, 38*(3), 114-121 110.1097/JES.1090b1013e3181e3728f.

Bouchard, C., Perusse, L., Chagnon, Y. C., Warden, C., & Ricquier, D. (1997). Linkage between markers in the vicinity of the Uncoupling Protein 2 hene and resting metabolic rate in humans. *Hum. Mol. Genet., 6*(11), 1887-1889.

Bozarth, M. A., & Wise, R. A. (1986). Involvement of the ventral tegmental dopamine system in opioid and psychomotor stimulant reinforcement. *NIDA Res Monogr, 67*, 190-196.

Bray, M. S., Hagberg, J. M., Parousse, L., Rankinen, T., Roth, S. M., Wolfarth, B., et al. (2009). The human gene map for performance and health-related fitness phenotypes: The 2006-2007 update. *Medicine & Science in Sports & Exercise, 41*(1), 35-73 10.1249/MSS.1240b1013e3181844179.

Brené, S., Bjørnebekk, A., Åberg, E., Mathé, A. A., Olson, L., & Werme, M. (2007). Running is rewarding and antidepressive. *Physiology & Behavior, 92*(1-2), 136-140.

Brown, P. L., Bae, D., & Kiyatkin, E. A. (2007). Relationships between locomotor activation and

alterations in brain temperature during selective blockade and stimulation of dopamine transmission. *Neuroscience, 145*(1), 335-343.

Burgess, C. R. (2010). Histamine and orexin in the control of arousal, locomotion, and motivation. *The Journal of neuroscience, 30*(8), 2810-2811.

Bye, A., Langaas, M., Hoydal, M. A., Kemi, O. J., Heinrich, G., Koch, L. G., et al. (2008). Aerobic capacity-dependent differences in cardiac gene expression. *Physiol. Genomics, 33*(1), 100-109.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience & Biobehavioral Reviews, 26*(3), 321-352.

Carlsson, M., & Carlsson, A. (1990). Interactions between glutamatergic and monoaminergic systems within the basal ganglia-implications for schizophrenia and Parkinson's disease. *Trends in Neurosciences, 13*(7), 272-274, C271-C274, 275-276.

Carlsson, S., Andersson, T., Lichtenstein, P., Michaelsson, K., & Ahlbom, A. (2006). Genetic effects on physical activity: Results from the Swedish Twin Registry. *Medicine & Science in Sports & Exercise, 38*(8), 1396-1401 1310.1249/1301.mss.0000228941.0000217034.c0000228941.

Chen, J.-X., Zhao, X., Yue, G.-X., & Wang, Z.-F. (2007). Influence of acute and chronic treadmill exercise on rat plasma lactate and brain NPY, L-ENK, DYN 1–13. *Cellular and Molecular Neurobiology, 27*(1), 1-10.

Choi, J.-K., Chen, Y. I., Hamel, E., & Jenkins, B. G. (2006). Brain hemodynamic changes mediated by dopamine receptors: Role of the cerebral microvasculature in dopamine-

mediated neurovascular coupling. *NeuroImage, 30*(3), 700-712.

Churchill, L., Klitenick, M. A., & Kalivas, P. W. (1998). Dopamine depletion reorganizes projections from the nucleus accumbens and ventral pallidum that mediate opioid-induced motor sactivity. *The Journal of neuroscience, 18*(19), 8074-8085.

Cooper, D. C. (2002). The significance of action potential bursting in the brain reward circuit. *Neurochemistry International, 41*(5), 333-340.

Cornford, E. M., Braun, L. D., Crane, P. D., & Oldendorf, W. H. (1978). Blood-brain barrier restriction of peptides and the low uptake of enkephalins. *Endocrinology, 103*(4), 1297-1303.

Cosgrove, K. P. (2003). Effects of a non-drug reinforcer, saccharin, on oral self-administration of phencyclidine in male and female rhesus monkeys. *Psychopharmacology, 170*(1), 9.

Cosgrove, K. P., Hunter, R. G., & Carroll, M. E. (2002). Wheel-running attenuates intravenous cocaine self-administration in rats: Sex differences. *Pharmacology Biochemistry and Behavior, 73*(3), 663-671.

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences, 25*(6), 295-301.

Curran, E. J., & Watson, S. J. (1995). Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: A double in situ hybridization study. *The Journal of Comparative Neurology, 361*(1), 57-76. doi: 10.1002/cne.903610106

Davis, J. M., Lamb, D. R., Lowy, M. T., Yim, G. K. W., & Malven, P. V. (1985). Opioid modulation of feeding behavior following forced swimming exercise in male rats. *Pharmacology Biochemistry and Behavior, 23*(5), 701-707.

Day, R., Lazure, C., Basak, A., Boudreault, A., Limperis, P., Dong, W., et al. (1998). Prodynorphin processing by Proprotein Convertase 2. *Journal of Biological Chemistry, 273*(2), 829-836.

De Moor, M. H. M., Posthuma, D., Hottenga, J.-J., Willemsen, G., Boomsma, D. I., & De Geus, E. J. C. (2007). Genome-wide linkage scan for exercise participation in Dutch sibling pairs. *Eur J Hum Genet, 15*(12), 1252-1259.

Depue, R. A., & Collins, P. F. (1999). Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. *Behav Brain Sci, 22*(3), 491-517; discussion 518-469.

Deutch, A. Y., & Cameron, D. S. (1992). Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. *Neuroscience, 46*(1), 49-56.

Di Ciano, P., Robbins, T. W., & Everitt, B. J. (2007). Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. *Neuropsychopharmacology, 33*(6), 1413-1425.

Dickerson, J. W., Hemmerle, A. M., Numan, S., Lundgren, K. H., & Seroogy, K. B. (2009). Decreased expression of ErbB4 and tyrosine hydroxylase mRNA and protein in the ventral midbrain of aged rats. *Neuroscience, 163*(1), 482-489. doi: 10.1016/j.neuroscience.2009.06.008

Dishman, R. K. (1997). Brain monoamines, exercise, and behavioral stress: animal models. *Medicine and science in sports and exercise, 29*(1), 63.

Dishman, R. K. (2008). Gene-Physical Activity Interactions in the Etiology of Obesity:

Behavioral Considerations. *Obesity, 16*(Supplement 3s), S60-S65.

Dishman, R. K., Berthoud, H.-R., Booth, F. W., Cotman, C. W., Edgerton, V. R., Fleshner, M. R., et al. (2006). Neurobiology of Exercise. *Obesity, 14*(3), 345-356.

Dishman, R. K., Dunn, A. L., Youngstedt, S. D., Davis, J. M., Burgess, M. L., Wilson, S. P., et al. (1996). Increased open field locomotion and decreased striatal GABAA binding after activity wheel running. *Physiology & Behavior, 60*(3), 699-705.

Dishman, R. K., & O'Connor, P. J. (2009). Lessons in exercise neurobiology: The case of endorphins. *Mental Health and Physical Activity, 2*(1), 4-9.

Dishman, R. K., Renner, K. J., Youngstedt, S. D., Reigle, T. G., Bunnell, B. N., Burke, K. A., et al. (1997). Activity wheel running reduces escape latency and alters brain monoamine levels after footshock. *Brain Research Bulletin, 42*(5), 399.

Doi, M., Yujnovsky, I., Hirayama, J., Malerba, M., Tirotta, E., Sassone-Corsi, P., & Borrelli, E. (2006). Impaired light masking in dopamine D2 receptor-null mice. [10.1038/nn1711]. *Nat Neurosci, 9*(6), 732-734. doi: http://www.nature.com/neuro/journal/v9/n6/suppinfo/nn1711_S1.html

Donahoo, W. T., Levine, J. A., & Melanson, E. L. (2004). Variability in energy expenditure and its components. *Curr Opin Clin Nutr Metab Care, 7*(6), 599-605.

Dunn, A. L., Reigle, T. G., Youngstedt, S. D., Armstrong, R. B., & Dishman, R. K. (1996). Brain norepinephrine and metabolites after treadmill training and wheel running in rats. *Medicine & Science in Sports & Exercise, 28*(2), 204-209.

Dunn, A. L., Trivedi, M. H., & O'Neal, H. A. (2001). Physical activity dose-response effects on outcomes of depression and anxiety. *Medicine & Science in Sports & Exercise, 33*(6),

S587-S597.

Eisenstein, S. A., & Holmes, P. V. (2007). Chronic and voluntary exercise enhances learning of conditioned place preference to morphine in rats. *Pharmacology Biochemistry and Behavior, 86*(4), 607-615.

Engber, T. M. (1992). Dopaminergic modulation of striatal neuropeptides: differential effects of D1 and D2 receptor stimulation on somatostatin, neuropeptide Y, neurotensin, dynorphin and enkephalin. *Brain Research, 581*(2), 261.

Eriksson, M., Rasmussen, F., & Tynelius, P. (2006). Genetic factors in physical activity and the equal environment assumption – the Swedish Young Male Twins Study. *Behavior Genetics, 36*(2), 238-247.

Fallon, J. H., & Leslie, F. M. (1986). Distribution of dynorphin and enkephalin peptides in the rat brain. *The Journal of Comparative Neurology, 249*(3), 293-336.

Fiatarone, M. A., Morley, J. E., Bloom, E. T., Benton, D., Makinodan, T., & Solomon, G. F. (1988). Endogenous opioids and the exercise-induced augmentation of natural killer cell activity. *J Lab Clin Med, 112*(5), 544-552.

Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., et al. (1997). Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. [10.1038/ng0397-269]. *Nat Genet, 15*(3), 269-272.

Foley, T., & Fleshner, M. (2008). Neuroplasticity of Dopamine Circuits After Exercise: Implications for Central Fatigue. *NeuroMolecular Medicine, 10*(2), 67-80.

Foley, T. E., Greenwood, B. N., Day, H. E. W., Koch, L. G., Britton, S. L., & Fleshner, M. (2006). Elevated central monoamine receptor mRNA in rats bred for high endurance

capacity: Implications for central fatigue. *Behavioural Brain Research, 174*(1), 132-142.

Foley, T. E., Greenwood, B. N., Koch, L. G., Britton, S. L., & Fleshner, M. (2005). Genetically selected high capacity runners have altered serotonergic and dopaminergic Receptor mRNA in the brain: 699 10:50 AM -11:05 AM. *Medicine & Science in Sports & Exercise, 37*(5), S133.

Garcia-Rill, E. (1986). The basal ganglia and the locomotor regions. *Brain Research Reviews, 11*(1), 47-63.

Garcia-Rill, E., Skinner, R. D., & Fitzgerald, J. A. (1985). Chemical activation of the mesecephalic locomotor region. *Brain research, 330*(1), 43-54.

Geisser, M. E., Wang, W., Smuck, M., Koch, L. G., Britton, S. L., & Lydic, R. (2008). Nociception before and after exercise in rats bred for high and low aerobic capacity. *Neuroscience Letters, 443*(1), 37-40.

Gerfen, C., Engber, T., Mahan, L., Susel, Z., Chase, T., Monsma, F., et al. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science, 250*(4986), 1429-1432.

Gerfen, C. R. (2000). Molecular effects of dopamine on striatal-projection pathways. *Trends in Neurosciences, 23*(Supplement 1), S64-S70.

Gonzalez, N. C., Howlett, R. A., Henderson, K. K., Koch, L. G., Britton, S. L., Wagner, H. E., et al. (2006). Systemic oxygen transport in rats artificially selected for running endurance. *Respiratory Physiology & Neurobiology, 151*(2-3), 141-150.

Gonzalez, N. C., Kirkton, S. D., Howlett, R. A., Britton, S. L., Koch, L. G., Wagner, H. E., et al. (2006). Continued divergence in VO2 max of rats artificially selected for running

endurance is mediated by greater convective blood O2 delivery. *J Appl Physiol, 101*(5), 1288-1296.

Graybiel, A. M. (1990). Neurotransmitters and neuromodulators in the basal ganglia. *Trends in Neurosciences, 13*(7), 244-254.

Greenwood, B. N., Foley, T. E., Le, T. V., Strong, P. V., Loughridge, A. B., Day, H. E. W., et al. (2010). Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behavioural Brain Research, 217*(2), 354-362.

Groenewegen, H. J. (2003). The basal ganglia and motor control. *Neural Plast, 10*(1-2), 107-120.

Grubbs, F. E., & Beck, G. (1972). Extension of sample sizes and percentage points for significance tests of outlying observations. *Technometrics, 14*(4), 847.

Gurney, K., Prescott, T. J., & Redgrave, P. (2001). A computational model of action selection in the basal ganglia. I. A new functional anatomy. *Biological Cybernetics, 84*(6), 401-410.

Haber, S. N., & Watson, S. J. (1985). The comparative distribution of enkephalin, dynorphin and substance P in the human globus pallidus and basal forebrain. *Neuroscience, 14*(4), 1011-1024.

Hagberg, J. M. (2010). Exercise genes? And no, not Levi's 501s! *J Appl Physiol, 109*(3), 619-620.

Hagberg, J. M., Rankinen, T., Loos, R. J., Perusse, L., Roth, S. M., Wolfarth, B., et al. (2011). Advances in exercise, fitness, and performance genomics in 2010. *Med Sci Sports Exerc, 43*(5), 743-752.

Halliday, G. M., & Törk, I. (1986). Comparative anatomy of the ventromedial mesencephalic tegmentum in the rat, cat, monkey and human. *The Journal of Comparative Neurology,*

252(4), 423-445.

Hanson, R. W., & Hakimi, P. (2008). Born to run; the story of the PEPCK-Cmus mouse. *Biochimie, 90*(6), 838-842. doi: 10.1016/j.biochi.2008.03.009

Harbach, H. W., & Hempelmann, G. (2005). Proopiomelanocortin and exercise. In W. J. Kraemer & A. D. Rogol (Eds.), *The Endocrine System and Exercise* (pp. 134-155). Malden, Ma.: Blackwell Publishing, Inc.

Hattori, S., Naoi, M., & Nishino, H. (1994). Striatal dopamine turnover during treadmill running in the rat: Relation to the speed of running. *Brain Research Bulletin, 35*(1), 41-49.

Hawkes, C. H. (1992). Endorphins: the basis of pleasure? *J Neurol Neurosurg Psychiatry, 55*(4), 247-250.

Herrero, M.-T., Barcia, C., & Navarro, J. (2002). Functional anatomy of thalamus and basal ganglia. *Child's Nervous System, 18*(8), 386-404.

Hersch, S. M., Ciliax, B. J., Gutekunst, C. A., Rees, H. D., Heilman, C. J., Yung, K. K., et al. (1995). Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J Neurosci, 15*(7 Pt 2), 5222-5237.

Heyes, M. P., Garnett, E. S., & Coates, G. (1988). Nigrostriatal dopaminergic activity is increased during exhaustive exercise stress in rats. *Life Sciences, 42*(16), 1537-1542.

Hoffmann, P., Terenius, L., & Thorén, P. (1990). Cerebrospinal fluid immunoreactive [beta]-endorphin concentration is increased by voluntary exercise in the spontaneously hypertensive rat. *Regulatory Peptides, 28*(2), 233-239.

Holmes, P. V. (1999). Olfactory bulbectomy increases prepro-enkephalin mRNA levels in the

ventral striatum in rats. *Neuropeptides, 33*(3), 206-211.

Holmes, P. V., Yoo, H. S., & Dishman, R. K. (2006). Voluntary exercise and clomipramine treatment elevate prepro-galanin mRNA levels in the locus coeruleus in rats. *Neuroscience Letters, 408*(1), 1-4.

Hook, S., Camberis, M., Prout, M., & Le Gros, G. (2003). Absence of preproenkephalin increases the threshold for T cell activation. *Journal of Neuroimmunology, 140*(1-2), 61-68.

Horvitz, J. C. (2002). Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. *Behavioural Brain Research, 137*(1-2), 65-74.

Hosseini, M., Alaei, H. A., Naderi, A., Sharifi, M. R., & Zahed, R. (2009). Treadmill exercise reduces self-administration of morphine in male rats. *Pathophysiology, 16*(1), 3.

Howlett, R. A., Kirkton, S. D., Gonzalez, N. C., Wagner, H. E., Britton, S. L., Koch, L. G., et al. (2009). Peripheral oxygen transport and utilization in rats following continued selective breeding for endurance running capacity. *J Appl Physiol, 106*(6), 1819-1825.

Hoydal, M. A., Wisloff, U., Kemi, O. J., & Ellingsen, O. (2007). Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training. *European Journal of Cardiovascular Prevention & Rehabilitation, 14*(6), 753-760 710.1097/HJR.1090b1013e3281eacef1091.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain research reviews, 56*(1), 27.

Ikemoto, S., Glazier, B. S., Murphy, J. M., & McBride, W. J. (1997). Role of dopamine D1 and

D2 receptors in the nucleus accumbens in mediating reward. *J. Neurosci., 17*(21), 8580-8587.

Iversen, I. H. (1993). Techniques for establishing schedules with wheel running as reinforcement in rats. *Journal of the Experimental Analysis of Behavior, 60*(1), 219.

Jackson, D. M., & Westlind-Danielsson, A. (1994). Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. *Pharmacology & Therapeutics, 64*(2), 291-370.

Jahn, K., Deutschländer, A., Stephan, T., Strupp, M., Wiesmann, M., & Brandt, T. (2004). Brain activation patterns during imagined stance and locomotion in functional magnetic resonance imaging. *NeuroImage, 22*(4), 1722-1731.

Johnson, P. I., & Stellar, J. R. (1994). Comparison of delta opiate receptor agonist induced reward and motor effects between the ventral pallidum and dorsal striatum. *Neuropharmacology, 33*(10), 1171-1182.

Johnson, R. A., & Mitchell, G. S. (2003). Exercise-induced changes in hippocampal brain-derived neurotrophic factor and neurotrophin-3: effects of rat strain. *Brain Research, 983*(1-2), 108-114.

Jónás, I., Schubert, K., Reijne, A., Scholte, J., Garland, T., Gerkema, M., . . . van Dijk, G. (2010). Behavioral traits are affected by selective breeding for increased wheel-running behavior in mice. *Behavior Genetics, 40*(4), 542-550. doi: 10.1007/s10519-010-9359-8

Jonsdottir, I. H. (2000). Neuropeptides and their interaction with exercise and immune function. *Immunol Cell Biol, 78*(5), 562-570.

Joosen, A. M., Gielen, M., Vlietinck, R., & Westerterp, K. R. (2005). Genetic analysis of physical activity in twins. *The American Journal of Clinical Nutrition, 82*(6), 1253-1259.

Joyce, E. M., & Koob, G. F. (1981). Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. *Psychopharmacology, 73*(4), 311-313.

Kelly, S. A., Nehrenberg, D. L., Peirce, J. L., Hua, K., Steffy, B. M., Wiltshire, T., et al. (2010). Genetic architecture of voluntary exercise in an advanced intercross line of mice. *Physiol. Genomics*, physiolgenomics.00028.02010.

Kieffer, B. L., & Gavériaux-Ruff, C. (2002). Exploring the opioid system by gene knockout. *Progress in Neurobiology, 66*(5), 285-306. doi: 10.1016/s0301-0082(02)00008-4

Knab, A. M., Bowen, R. S., Hamilton, A. T., Gulledge, A. A., & Lightfoot, J. T. (2009). Altered dopaminergic profiles: Implications for the regulation of voluntary physical activity. *Behavioural Brain Research, 204*(1), 147-152.

Knab, A. M., & Lightfoot, J. T. (2010). Does the difference between physically active and couch potato lie in the dopamine system? *Int J Biol Sci, 6*(2), 133-150.

Knutson, B., & Gibbs, S. (2007). Linking nucleus accumbens dopamine and blood oxygenation. *Psychopharmacology, 191*(3), 813-822.

Koch, L. G., & Britton, S. L. (2001). Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiol. Genomics, 5*(1), 45-52.

Koch, L. G., & Britton, S. L. (2008). Aerobic metabolism underlies complexity and capacity. *The Journal of Physiology, 586*(1), 83-95.

Koch, L. G., & Britton, S. L. (2008). Development of Animal Models to Test the Fundamental Basis of Gene-Environment Interactions. *Obesity, 16*(Supplement 3s), S28-S32.

Koepp, M. J., Hammers, A., Lawrence, A. D., Asselin, M. C., Grasby, P. M., & Bench, C. J.

(2009). Evidence for endogenous opioid release in the amygdala during positive emotion. *NeuroImage, 44*(1), 252-256.

Koga, E., & Momiyama, T. (2000). Presynaptic dopamine D2-like receptors inhibit excitatory transmission onto rat ventral tegmental dopaminergic neurones. *The Journal of Physiology, 523*(1), 163-173.

Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends in Pharmacological Sciences, 13*, 177-184.

Koob, G. F., & Le Moal, M. (2008). Addiction and the brain antireward system. *Annual review of psychology, 59*, 29-53.

Koob, G. F., Riley, S. J., Smith, S. C., & Robbins, T. W. (1978). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. *Journal of Comparative and Physiological Psychology, 92*(5), 917-927.

Kruger, J., Ham, S. A., & Kohl, H. W., III. (2005). Trends in leisure-time physical inactivity by age, sex, and race/ethnicity-United States, 1994-2004. *Morbidity and mortality weekly report, 54*(39), 991.

Kruger, J., Kohl, H. W., III, & Miles, I. J. (2007). Prevalence of regular physical activity among adults-United States, 2001 and 2005. *Morbidity and mortality weekly report, 56*(46), 1209.

Lajtha, A., Galoyan, A., Besedovsky, H., & Jessop, D. (2008). Neuropeptides in the Immune System: Mediators of Stress and Inflammation *Handbook of Neurochemistry and Molecular Neurobiology* (pp. 19-35): Springer US.

Land, B. B., Bruchas, M. R., Schattauer, S., Giardino, W. J., Aita, M., Messinger, D., . . . Chavkin, C. (2009). Activation of the kappa opioid receptor in the dorsal raphe nucleus mediates the aversive effects of stress and reinstates drug seeking. *Proceedings of the National Academy of Sciences, 106*(45), 19168-19173. doi: 10.1073/pnas.0910705106

Lauderdale, D. S., Fabsitz, R., Meyer, J. M., Sholinsky, P., Ramakrishnan, V., & Goldberg, J. (1997). Familial determinants of moderate and intense physical activity: a twin study. *Medicine & Science in Sports & Exercise, 29*(8), 1062-1068.

Le Moine, C. (1990). Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proceedings of the National Academy of Sciences of the United States of America, 87*(1), 230.

Leamy, L., Pomp, D., & Lightfoot, J. (2010). A search for quantitative trait loci controlling within-individual variation of physical activity traits in mice. *BMC Genetics, 11*(1), 83.

Leknes, S., & Tracey, I. (2008). A common neurobiology for pain and pleasure. [10.1038/nrn2333]. *Nat Rev Neurosci, 9*(4), 314-320.

Lenard, N. R., & Berthoud, H.-R. (2008). Central and Peripheral Regulation of Food Intake and Physical Activity: Pathways and Genes. *Obesity, 16*(Supplement 3s), S11-S22.

Lessard, S. J., Rivas, D. A., Chen, Z.-P., van Denderen, B. J., Watt, M. J., Koch, L. G., et al. (2009). Impaired skeletal muscle {beta}-adrenergic activation and lipolysis are associated with whole-body insulin resistance in rats bred for low intrinsic exercise capacity. *Endocrinology, 150*(11), 4883-4891.

Lett, B. T. (2000). Pairings of a distinctive chamber with the aftereffect of wheel running produce conditioned place preference* 1,* 2. *Appetite, 34*(1), 87.

Li, S. J., Jiang, H. K., Stachowiak, M. S., Hudson, P. M., Owyang, V., Nanry, K., et al. (1990). Influence of nigrostriatal dopaminergic tone on the biosynthesis of dynorphin and enkephalin in rat striatum. *Molecular Brain Research, 8*(3), 219-225.

Lighfoot, J. T., Leamy, L. J., Pomp, D., Turner, M. J., Fodor, A. A., Knab, A., et al. (2010). Strain screen and haplotype association mapping of wheel running in inbred mouse strains. *J Appl Physiol*, japplphysiol.00525.02010.

Lightfoot, J. T. (2011a). Can you be born a couch potato? The genomic regulation of physical activity. In L. S. Pescatello & S. M. Roth (Eds.), *Exercise Genomics* (pp. 45-72): Humana Press.

Lightfoot, J. T. (2011b). Current understanding of the genetic basis for physical activity. *The Journal of Nutrition, 141*(3), 526-530.

Lightfoot, J. T., Hamilton, A., & Moore-Harrison, T. (2010). Differential gene expression in high and low active animals. *Medicine & Science in Sports & Exercise, 42*(10), 99 10.1249/1201.MSS.0000389463.0000393642.0000389473.

Lightfoot, J. T., Leamy, L., Pomp, D., Turner, M. J., Fodor, A. A., Knab, A., et al. (2010). Strain screen and haplotype association mapping of wheel running in inbred mouse strains. *Journal of Applied Physiology, 109*(3), 623-634.

Lightfoot, J. T., Turner, M. J., Daves, M., Vordermark, A., & Kleeberger, S. R. (2004). Genetic influence on daily wheel running activity level. *Physiol. Genomics, 19*(3), 270-276.

Lightfoot, J. T., Turner, M. J., Knab, A. K., Jedlicka, A. E., Oshimura, T., Marzec, J., et al. (2007). Quantitative trait loci associated with maximal exercise endurance in mice. *Journal of Applied Physiology, 103*(1), 105-110.

Lightfoot, J. T., Turner, M. J., Pomp, D., Kleeberger, S. R., & Leamy, L. J. (2008). Quantitative trait loci for physical activity traits in mice. *Physiol. Genomics, 32*(3), 401-408.

Lu, L., Grimm, J. W., Shaham, Y., & Hope, B. T. (2003). Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. *Journal of Neurochemistry, 85*(6), 1604-1613.

Lu, X. Y., Behnam Ghasemzadeh, M., & Kalivas, P. W. (1997). Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience, 82*(3), 767-780.

Lujan, H. L., Britton, S. L., Koch, L. G., & DiCarlo, S. E. (2006). Reduced susceptibility to ventricular tachyarrhythmias in rats selectively bred for high aerobic capacity. *Am J Physiol Heart Circ Physiol, 291*(6), H2933-2941.

Luo, M. C., Chen, Q., Ossipov, M. H., Rankin, D. R., Porreca, F., & Lai, J. (2008). Spinal dynorphin and bradykinin receptors maintain inflammatory hyperalgesia. *J Pain, 9*(12), 1096-1105.

MacRae, P. G., Spirduso, W. W., Cartee, G. D., Farrar, R. P., & Wilcox, R. E. (1987). Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolite levels. *Neuroscience Letters, 79*(1-2), 138-144.

MacRae, P. G., Spirduso, W. W., Walters, T. J., Farrar, R. P., & Wilcox, R. E. (1987). Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolites in presenescent older rats. *Psychopharmacology, 92*(2), 236-240.

Maia, J. A. R., Thomis, M., & Beunen, G. (2002). Genetic factors in physical activity levels: A twin study. *American journal of preventive medicine, 23*(2), 87-91.

Mansour, A., Fox, C. A., Akil, H., & Watson, S. J. (1995). Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends in Neurosciences, 18*(1), 22-29.

Marsden, C. D., & Obeso, J. A. (1994). The functions of the basal ganglia and the paradox of stereotaxic surgery in Parkinson's disease. *Brain, 117 (Pt 4)*, 877-897.

Mathes, W. F., Nehrenberg, D. L., Gordon, R., Hua, K., Garland Jr, T., & Pomp, D. (2010). Dopaminergic dysregulation in mice selectively bred for excessive exercise or obesity. *Behavioural Brain Research, 210*(2), 155-163.

McClung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2004). [Delta]FosB: a molecular switch for long-term adaptation in the brain. *Molecular Brain Research, 132*(2), 146-154.

McNab, F., Varrone, A., Farde, L., Jucaite, A., Bystritsky, P., Forssberg, H., et al. (2009). Changes in cortical dopamine D1 receptor binding associated with cognitive training. *Science, 323*(5915), 800-802.

McRitchie, D. A., Hardman, C. D., & Halliday, G. M. (1996). Cytoarchitectural distribution of calcium binding proteins in midbrain dopaminergic regions of rats and humans. *J Comp Neurol, 364*(1), 121-150.

Meeusen, R., & De Meirleir, K. (1995). Exercise and brain neurotransmission. *Sports Med, 20*(3), 160-188.

Missale, C., Nash, S. R., Robinson, S. W., jaber, M., & Caron, M. G. (1998). Dopamine receptors: from structure to function. *Physiol. Rev., 78*(1), 189-225.

Mogenson, G.J. (1987) Limbic-motor integration. *Progress in Psychobiology and Physiological*

Psychology, 12, 117-170.

Moraska, A., Deak, T., Spencer, R. L., Roth, D., & Fleshner, M. (2000). Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 279*(4), R1321-R1329.

Murray, P. S., Groves, J. L., Pettett, B. J., Britton, S. L., Koch, L. G., Dishman, R. K., & Holmes, P. V. (2010). Locus coeruleus galanin expression is enhanced after exercise in rats selectively bred for high capacity for aerobic activity. *Peptides, 31*(12), 2264-2268.

Mustelin, L., Silventoinen, K., Pietilainen, K., Rissanen, A., & Kaprio, J. (2009). Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. *Int J Obes, 33*(1), 29-36.

Neeper, S. A., Gomez-Pinilla, F., Choi, J., & Cotman, C. (1995). Exercise and brain neurotrophins. *Nature, 373*(6510), 109.

Nestler, E. J. (1992). Molecular mechanisms of drug addiction. *The Journal of neuroscience, 12*(7), 2439-2450.

Nestler, E. J., Barrot, M., & Self, D. W. (2001). Delta FosB: A sustained molecular switch for addiction. *PNAS, 98*(20), 11042-11046.

Nieto, M. M., Guen, S. L. E., Kieffer, B. L., Roques, B. P., & Noble, F. (2005). Physiological control of emotion-related behaviors by endogenous enkephalins involves essentially the delta opioid receptors. *Neuroscience, 135*(2), 305-313.

Novak C.M., Escande C., Burghardt P.R., Zhang M., Barbosa M.T., Chini E.N., Britton S.L., Koch L.G., Akil H., Levine J.A., 2010. Spontaneous activity, economy of activity, and

resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity. Hormones and Behavior. 58(3), 355-367.

Nybo, L., & Secher, N. H. (2004). Cerebral perturbations provoked by prolonged exercise. *Progress in Neurobiology, 72*(4), 223-261.

Ouchi, Y., Yoshikawa, E., Futatsubashi, M., Okada, H., Torizuka, T., & Sakamoto, M. (2002). Effect of simple motor performance on regional dopamine release in the striatum in Parkinson disease patients and healthy subjects: A positron emission tomography study. *J Cereb Blood Flow Metab, 22*(6), 746-752.

Parent, A., & Hazrati, L.-N. (1995). Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Research Reviews, 20*(1), 91-127.

Perrine, S. A., Sheikh, I. S., Nwaneshiudu, C. A., Schroeder, J. A., & Unterwald, E. M. (2008). Withdrawal from chronic administration of cocaine decreases delta opioid receptor signaling and increases anxiety- and depression-like behaviors in the rat. *Neuropharmacology, 54*(2), 355-364.

Personius, K. E., Jayaram, A., Krull, D., Brown, R., Xu, T., Han, B., . . . Welle, S. (2010). Grip force, EDL contractile properties, and voluntary wheel running after postdevelopmental myostatin depletion in mice. *Journal of Applied Physiology, 109*(3), 886-894. doi: 10.1152/japplphysiol.00300.2010

Phillips, A. G. (1980). Reinforcing effects of morphine microinjection into the ventral tegmental area. *Pharmacology, biochemistry and behavior, 12*(6), 965.

Phillipson, O. T. (1979). The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of tsai in the rat. *The Journal of Comparative Neurology, 187*(1), 85-98.

Pollack, A. E., & Wooten, G. F. (1992). Differential regulation of striatal preproenkephalin mRNA by D1 and D2 dopamine receptors. *Molecular Brain Research, 12*(1-3), 111-119.

Primeaux, S. D., & Holmes, P. V. (2000). Olfactory bulbectomy increases met-enkephalin- and neuropeptide-Y-like immunoreactivity in rat limbic structures. *Pharmacology Biochemistry and Behavior, 67*(2), 331-337.

Puntschart, A., Wey, E., Jostarndt, K., Vogt, M., Wittwer, M., Widmer, H. R., et al. (1998). Expression of fos and jun genes in human skeletal muscle after exercise. *Am J Physiol Cell Physiol, 274*(1), C129-137.

Rada, P., Barson, J. R., Leibowitz, S. F., & Hoebel, B. G. (2009). Opioids in the hypothalamus control dopamine and acetylcholine levels in the nucleus accumbens. *Brain Research, 1312*, 1-9.

Rankinen, T., Roth, S. M., Bray, M. S., Loos, R., Perusse, L., Wolfarth, B., et al. (2010). Advances in exercise, fitness, and performance genomics. *Med Sci Sports Exerc, 42*(5), 835-846.

Ravussin, E., & Bogardus, C. (2000). Energy balance and weight regulation: genetics versus environment. *Br J Nutr, 83 Suppl 1*, S17-20.

Rhodes, J. S., Gammie, S. C., & Garland, T. (2005). Neurobiology of mice selected for high voluntary wheel-running activity. *Integrative and Comparative Biology, 45*(3), 438-455.

Rhodes, J. S., & Garland, T. (2003). Differential sensitivity to acute administration of Ritalin, apormorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology, 167*(3), 242-250.

Rhodes, J. S., Hosack, G. R., Girard, I., Kelley, A. E., Mitchell, G. S., & Garland, T., Jr. (2001).

Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology (Berl), 158*(2), 120-131.

Rhodes, J. S., van Praag, H., Jeffrey, S., Girard, I., Mitchell, G. S., Garland, T., et al. (2003). Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behavioral Neuroscience, 117*(5), 1006-1016.

Robbins, T. W., Cador, M., Taylor, J. R., & Everitt, B. J. (1989). Limbic-striatal interactions in reward-related processes. *Neuroscience & Biobehavioral Reviews, 13*(2-3), 155-162.

Robbins, T. W., & Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology, 6*(2), 228-236.

Rossier, J., Bayon, A., Vargo, T. M., Ling, N., Guillemin, R., & Bloom, F. (1977). Radioimmunoassay of brain peptides: Evaluation of a methodology for the assay of [beta]-endorphin and enkephalin. *Life Sciences, 21*(6), 847-852.

Rougé-Pont, F., Deroche, V., Moal, M. L., & Piazza, P. V. (1998). Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *European Journal of Neuroscience, 10*(12), 3903-3907.

Sakuma, Y. (2008). Neural substrates for sexual preference and motivation in the female and male rat. *Annals of the New York Academy of Sciences, 1129*(1), 55-60. doi: 10.1196/annals.1417.009

Sarbadhikari, S. N., & Saha, A. K. (2006). Moderate exercise and chronic stress produce counteractive effects on different areas of the brain by acting through various

neurotransmitter receptor subtypes: a hypothesis. *Theor Biol Med Model, 3*, 33. doi: 10.1186/1742-4682-3-33

Salamone, J. D., & Correa, M. (2002). Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behavioural Brain Research, 137*(1-2), 3-25.

Salamone, J. D., Correa, M., Mingote, S., & Weber, S. M. (2003). Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: Implications for studies of natural motivation, psychiatry, and drug Abuse. *Journal of Pharmacology and Experimental Therapeutics, 305*(1), 1-8.

Scarpace, P. J., Matheny, M., & Zhang, Y. (2010). Wheel running eliminates high-fat preference and enhances leptin signaling in the ventral tegmental area. *Physiology & Behavior, 100*(2), 173-179. doi: 10.1016/j.physbeh.2010.02.017

Scheurink, A. J. W., Boersma, G. J., Nergårdh, R., & Södersten, P. (2010). Neurobiology of hyperactivity and reward: Agreeable restlessness in Anorexia Nervosa. *Physiology & Behavior, 100*(5), 490-495.

Siegel, A., Zalcman, S. S., Fleshner, M., Kennedy, S. L., Johnson, J. D., Day, H. E. W., et al. (2009). Exercise and stress resistance: Neural-immune mechanisms. *The Neuroimmunological Basis of Behavior and Mental Disorders* (pp. 87-107): Springer US.

Simon, P., Fehrenbach, E., & Niess, A. M. (2006). Regulation of immediate early gene expression by exercise: short cuts for the adaptation of immune function. *Exerc Immunol Rev, 12*, 112-131.

Simonen, R. L., Rankinen, T., Perusse, L., Rice, T., Rao, D. C., Chagnon, Y., et al. (2003).

Genome-wide linkage scan for physical activity levels in the Quebec Family Study. *Medicine & Science in Sports & Exercise, 35*(8), 1355-1359.

Smith, J., Feldman, J., & Schmidt, B. (1988). Neural mechanisms generating locomotion studied in mammalian brain stem- spinal cord in vitro. *The FASEB Journal, 2*(7), 2283-2288.

Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proceedings of the National Academy of Sciences*.

Smith, M. A. (2008). Aerobic exercise decreases the positive-reinforcing effects of cocaine. *Drug and alcohol dependence, 98*(1-2), 129.

Sommers, K., Loots, J. M., Simpson, S. F., Meyer, E. C., Dettweiler, A., & Human, J. l. R. (1990). Circulating met-enkephalin in trained athletes during rest, exhaustive treadmill exercise and marathon running. *European Journal of Clinical Pharmacology, 38*(4), 391-392.

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences, 89*(6), 2046-2050.

Spargo, F. J., McGee, S. L., Dzamko, N., Watt, M. J., Kemp, B. E., Britton, S. L., et al. (2007). Dysregulation of muscle lipid metabolism in rats selectively bred for low aerobic running capacity. *Am J Physiol Endocrinol Metab, 292*(6), E1631-1636.

Stubbe, J. H., Boomsma, D. I., Vink, J. M., Cornes, B. K., Martin, N. G., Skytthe, A., et al. (2006). Genetic influences on exercise participation in 37,051 twin pairs from seven countries. *PLoS ONE, 1*(1), e22.

Swallow, J. G., Carter, P. A., & Garland, T. (1998). Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics, 28*(3), 227-237.

Takakusaki, K., Oohinata-Sugimoto, J., Saitoh, K., & Habaguchi, T. (2004). Role of basal ganglia-brainstem systems in the control of postural muscle tone and locomotion. In D. G. S. Shigemi Mori & W. Mario (Eds.), *Progress in Brain Research* (Vol. Volume 143, pp. 231-237): Elsevier.

Tekumalla, P. K., Calon, F., Rahman, Z., Birdi, S., Rajput, A. H., Hornykiewicz, O., et al. (2001). Elevated levels of [Delta]FosB and RGS9 in striatum in Parkinson's disease. *Biological Psychiatry, 50*(10), 813-816.

Tong, L., Shen, H., Perreau, V. M., Balazs, R., & Cotman, C. W. (2001). Effects of Exercise on Gene-Expression Profile in the Rat Hippocampus. *Neurobiology of Disease, 8*(6), 1046-1056.

Torregrossa, M. M., Jutkiewicz, E. M., Mosberg, H. I., Balboni, G., Watson, S. J., & Woods, J. H. (2006). Peptidic delta opioid receptor agonists produce antidepressant-like effects in the forced swim test and regulate BDNF mRNA expression in rats. *Brain Research, 1069*(1), 172-181.

Torvinen, S., Silvennoinen, M., Gröhn, O., Närväinen, J., Tuunanen, P., Piitulainen, H., et al. (2010). Rats bred for low aerobic capacity become rapidly fatigued and have slow metabolic recovery after stimulated muscle contractions. *Medicine & Science in Sports & Exercise, 42*(10), 54 10.1249/1201.MSS.0000389347.0000374343.d0000389349.

Tran, A. H., Tamura, R., Uwano, T., Kobayashi, T., Katsuki, M., Matsumoto, G., et al. (2002). Altered accumbens neural response to prediction of reward associated with place in

dopamine D2 receptor knockout mice. *Proceedings of the National Academy of Sciences of the United States of America, 99*(13), 8986-8991.

Ungerstedt, U., & Arbuthnott, G. W. (1970). Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Research, 24*(3), 485-493.

Vallone, D., Picetti, R., & Borrelli, E. (2000). Structure and function of dopamine receptors. *Neuroscience & Biobehavioral Reviews, 24*(1), 125-132.

Van Bockstaele, E. J. (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Research, 682*(1-2), 215.

Van Hoomissen, J. D., Chambliss, H. O., Holmes, P. V., & Dishman, R. K. (2003). Effects of chronic exercise and imipramine on mRNA for BDNF after olfactory bulbectomy in rat. *Brain research, 974*(1-2), 228-235.

van Praag, H. (2008). Neurogenesis and exercise: past and future directions. *Neuromolecular Med, 10*(2), 128-140.

Vargas-Pérez, H., Borrelli, E., & Díaz, J.-L. (2004). Wheel running use in dopamine D2L receptor knockout mice. *Neuroscience Letters, 366*(2), 172-175.

Wang, G.-J., Volkow, N. D., Thanos, P. K., & Fowler, J. S. (2009). Imaging of brain dopamine pathways: Implications for understanding obesity. *Journal of Addiction Medicine, 3*(1), 8-18 10.1097/ADM.1090b1013e31819a31886f31817.

Warburton, D. E. R., Nicol, C. W., & Bredin, S. S. D. (2006). Health benefits of physical activity: the evidence. *CMAJ, 174*(6), 801-809.

Waters, R. P., Renner, K. J., Pringle, R. B., Summers, C. H., Britton, S. L., Koch, L. G., et al.

(2008). Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. *Physiol Behav, 93*(4-5), 1044-1054.

Werme, M., Lindholm, S., Thorén, P., Franck, J., & Brené, S. (2002a). Running increases ethanol preference. *Behavioural Brain Research, 133*(2), 301-308.

Werme, M., Messer, C., Olson, L., Gilden, L., Thoren, P., Nestler, E. J., et al. (2002b). Delta FosB Regulates Wheel Running. *J. Neurosci., 22*(18), 8133-8138.

Werme, M., Thorén, P., Olson, L., & Brené, S. (2000). Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. *European Journal of Neuroscience, 12*(8), 2967-2974.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat Rev Neurosci, 5*(6), 483-494.

Wise, R. A. (2009). Roles for nigrostriatal--not just mesocorticolimbic--dopamine in reward and addiction. *Trends in Neurosciences, 32*(10), 517-524.

Wise, R. A., & Schwartz, H. V. (1981). Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmacol Biochem Behav, 15*(4), 655-656.

Wisloff, U., Najjar, S. M., Ellingsen, O., Haram, P. M., Swoap, S., Al-Share, Q., et al. (2005). Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science, 307*(5708), 418-420.

Xu, M., Moratalla, R., Gold, L. H., Hiroi, N., Koob, G. F., Graybiel, A. M., et al. (1994). Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. *Cell, 79*(4), 729-742.

Young, W. S., Bonner, T. I., & Brann, M. R. (1986). Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. *Proceedings of the National*

Academy of Sciences, 83(24), 9827-9831.

Yung, K.K.L., Bolam, J. P., Smith, A.D., Hersch, S. M., Ciliax, B.J. & Levey, A.I. (1995) Immunocytochemical localization of D1 and D2 dopamine receptors in the rat: Light and electron microscopy. *Neuroscience, 65*(3), 709-730.