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SYMPOSIUM

Hormones and the Evolution of Complex Traits: Insights from Artificial Selection on Behavior

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Synopsis Although behavior may often be a fairly direct target of natural or sexual selection, it cannot evolve without changes in subordinate traits that cause or permit its expression. In principle, changes in endocrine function could be a common mechanism underlying behavioral evolution because they are well positioned to mediate integrated responses to behavioral selection. More specifically, hormones can influence both motivational (e.g., brain) and performance (e.g., muscles) components of behavior simultaneously and in a coordinated fashion. If the endocrine system is often "used" as a general mechanism to effect responses to selection, then correlated responses in other aspects of behavior, life history, and organismal performance (e.g., locomotor abilities) should commonly occur because any cell with appropriate receptors could be affected. Ways in which behavior coadapts with other aspects of the phenotype can be studied directly through artificial selection and experimental evolution. Several studies have targeted rodent behavior for selective breeding and reported changes in other aspects of behavior, life history, and lower-level effectors of these organismal traits, including endocrine function. One example involves selection for high levels of voluntary wheel running, one aspect of physical activity, in four replicate High Runner (HR) lines of mice. Circulating levels of several hormones (including insulin, testosterone, thyroxine, triiodothyronine) have been characterized, three of which—corticosterone, leptin, and adiponectin—differ between HR and control lines, depending on sex, age, and generation. Potential changes in circulating levels of other behaviorally and metabolically relevant hormones, as well as in other components of the endocrine system (e.g., receptors), have yet to be examined. Overall, results to date identify promising avenues for further studies on the endocrine basis of activity levels.

Introduction

Behavior may often be a fairly direct target of natural or sexual selection; however, it cannot evolve without changes in subordinate traits that cause or permit its expression. Because of their diverse effects on both neural and peripheral aspects of behavior, changes in endocrine function could be a common mechanism underlying behavioral evolution. If so, then correlated responses in other aspects of behavior, life history (Dantzer et al. 2017), and organismal performance (e.g., locomotor abilities) should commonly occur because any cell with appropriate receptors could be affected. At the same time, because hormones are likely to affect multiple traits, including through early-life parental effects (e.g., see Garland et al. (2017)), they might be "used" routinely by selection to achieve (adaptively) correlated changes (Cox et al. 2017). Nevertheless, the seminal papers in modern evolutionary physiology scarcely mentioned the endocrine system (Feder 1987; Garland and Carter 1994; Feder et al. 2000). Evolutionary endocrinology has thus developed somewhat separately from the rest of evolutionary physiology (Zera and Zhang 1995; Zera et al. 2007; Nepomnaschy et al. 2009; Adkins-Regan 2012; Cox et al. 2017; Dantzer et al. 2017). Although hybrid fields always run the risk of settling into a pattern of "choose one from column A and one from Column B," evolutionary endocrinology can move beyond this trap by addressing emergent questions that truly integrate across disciplines and generally would not have been addressed

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Table 1 Evolutionary endocrinology as a hybrid field that addresses emergent questions

Field	Evolutionary Biology	Endocrinology
Within-field Questions	How do populations change over time? How can we use "model organisms" to elucidate basic genetic and evolutionary principles? Relatively how important are natural selection, sexual selection, and random genetic drift in shaping biological diversity?	How does the endocrine system work? How can we use "model organisms" to elucidate basic endocrine principles? Where is the line between "normal" individual variation and pathology? How do we promote health and cure disease?
Emergent Questions	How does the endocrine system constrain or facilitate behavioral evolution? Which cases of pleiotropic gene action can be attributed to genetic variation in components of the endocrine system? What are the most common endocrine mechanisms that respond to selection on behavior or life-history traits? Which correlated responses to selection on behavior represent non-adaptive or maladaptive byproducts of endocrine function?	

by pure practitioners of either parental field (Table 1).

Ways in which behavior coadapts with other aspects of the phenotype can be investigated with various approaches, such as through phylogenetically based comparative studies (Garland et al. 2005; Rezende and Diniz-Filho 2012). Comparative studies, however, are necessarily correlational and historical in nature (Abbott et al. 2003; Goymann et al. 2004) and cannot reveal the detailed generation-to-generation changes that occur in response to selection. Quantitative-genetic analyses and studies of selection in the wild can reveal the patterns of genetic and phenotypic variation in endocrine components that form the basis for individual variation and covariation as well as the higher-level phenotypes on which natural and sexual selection act in sex-, population- and context-specific ways (Shire 1976; Storz et al. 2015; Cox et al. 2017; Dantzer et al. 2017). Beyond this, various types of phenoytpic engineering, e.g., by manipulating circulating levels of particular hormones, can help to elucidate both functional relations and selective importance (e.g., Ketterson et al. 1996; Ketterson et al. 2009; Cox et al. 2014; Dantzer et al. 2017). Finally, phenotypic evolution can be studied directly with selection experiments and experimental evolution (Swallow and Garland 2005; Garland and Rose 2009; Kawecki et al. 2012; Storz et al. 2015). Most germane to this paper, several studies have targeted rodent behavior for selective breeding and reported changes in other aspects of behavior, life history, and lower-level effectors of these organismal traits, including endocrine function (Hyde 1981; Rhodes and Kawecki 2009; Swallow et al. 2009).

In this overview, we will first consider the nature and evolution of complex traits, which generally include motivated behaviors. We then provide a very brief summary of the endocrine system and some of its more easily understood components. Next, we consider some key points about how natural selection typically acts on behavior. We then turn to examples of artificial selection on behavior, as well as one example of selection that was imposed directly on an endocrine function. We provide a summary of key endocrine findings from a long-term experiment in which mice are bred for high levels of voluntary wheel running. Finally, we draw some conclusions regarding hormones and the evolution of behavior, emphasizing what we have learned from rodent selection experiments.

Complex traits

So-called "complex traits" can be defined in various ways. First, they occur at relatively high levels of biological organization, including life-history traits (e.g., reproductive mode, such as viviparity) and most behaviors (one might exclude simple neuromuscular reflex arcs). Complex traits necessarily comprise many subordinate traits (also known as endophenotypes, e.g.: Gottesman and Gould 2003). As a behavioral example, consider ingestion (food intake). The "decision" by an animal to ingest or reject a given substance follows from sensory inputs (e.g., smell, sight, taste), rapidly integrated in the central nervous system, then leading to motor output (Berthoud 2007). The decision is influenced by a variety of external factors, such as whether the food item offers any resistance or is noxious and whether the organism perceives any predation risk while dealing with the potential food item. Internal to the organism, the endocrine systems can influence ingestion behavior at several levels, including in

relation to internal states of energy balance, hunger, appetite, and affect (Magni et al. 2009; Hoskins and Volkoff 2012).

As an even more complex behavioral example, consider voluntary activity levels. In the wild, this relates to, for example, use of the home range; searching for mates, food, and other resources; patrolling territory; and migration (Feder et al. 2010). For human beings in Western industrialized societies, a large part of activity levels may relate to voluntary exercise behavior, or the lack thereof. Even (or perhaps especially) within the built environment of human societies, the control of exercise behavior is exceedingly complex and involves numerous internal and external factors, plus interactive effects (Dishman et al. 2006; Garland et al. 2011b).

A second characteristic of complex traits is that they often exhibit emergent properties, which is to say that what they do or how they work is not entirely predictable from knowledge of how their lower-level components function (Novikoff 1945; Lobo 2008). Commonly cited examples in the behavioral realm include consciousness, bird flocks, social behavior, and the functionality of bee hives.

Third, complex traits are characterized by modularity (Csete and Doyle 2002). This means that components of the overall trait can often function or evolve somewhat independently. For example, with adequate oxygenation and nutrient supply, an isolated heart or skeletal muscle can contract, generate force, and perform work. Similarly, components of ingestive behavior can function independently, and often as components of other complex behaviors (e.g., smell, motor output). The modules of complex traits are integrated in both form and function when in situ, and take on greater roles when so integrated.

Fourth, complex traits are affected by many genes and environmental factors, plus interactions of genes with genes, environmental factors with other environmental factors, and gene-by-environment interactions. Moreover, epigenetic processes can affect many components of complex traits. "Epigenetic" in modern parlance generally refers to changes caused by modification of gene expression rather than alteration of the genetic code itself. Sometimes the definition is restricted to heritable changes in gene expression that do not involve changes to the underlying DNA sequence, such as may occur by DNA methylation (Waterland and Garza 1999; Burggren and Crews 2014; Waterland 2014; Garland et al. 2017) or histone modification (Lennartsson and Ekwall 2009).

A final point about complex traits is that several of them may share specific functional components. This will obviously be true for any of the major "organ systems," and perhaps especially true for the endocrine system, given that particular hormones and hormone receptors often play a role in multiple behaviors or physiological processes (see below; Cox et al. 2017; Schwartz and Bronikowski 2017). For example, repeated evolutionary patterns of duplication and divergence of genes for steroid hormone receptors have led to pronounced changes in the endocrine control of reproductive morphology, physiology, and behavior (e.g., see Thornton 2001; Baker 2004; Young and Crews 1995; Dean and Thornton 2007; Bridgham et al. 2009). These and other deep and diverse phylogenetic patterns of endocrine origins would lead one to expect considerable pleiotropic gene action both among the components of a given complex trait and across components—and hence across different complex traits.

Given all of the foregoing characteristics of complex traits, it should be of little surprise that their evolutionary history is generally complicated and multifactorial. A good example of this is the evolution of lactation strategies in pinnipeds, as reviewed by Schulz and Bowen (2005). They describe (e.g., see their Figure 4) complex interrelationships of environmental factors (e.g., temperature, predation regime), body size, rates of energy expenditure, fat storage, and so forth, with many of the behavioral and physiological aspects under at least partial endocrine control, although that point is not mentioned in the article.

Another example of the multifaceted evolution of a complex trait involves endothermy. In particular, the evolution of mammalian (and avian) endothermy has long been of interest in comparative, ecological, and evolutionary physiology (Garland and Carter 1994), but so far has escaped much scrutiny from evolutionary endocrinologists (Alberts and Pickler 2012; Little and Seebacher 2014). Kemp (2006) views the origin of mammalian endothermy as a paradigm for the evolution of complex traits. His Figure 3 depicts "interrelationships of the structures and functions responsible for or affected by endothermic temperature physiology of a mammal," and also acknowledges the role of endocrine regulation. Koteja (2000) presents a model involving energy assimilation and parental care in the evolution of endothermy, but does not mention the endocrine system. On the other hand, Farmer (2000) does emphasize the potential importance of thyroid (and other) hormones in her model for the evolution of endothermy in relation to parental care. A recent review also promotes possible roles of the endocrine system, especially thyroid hormones (Nespolo et al.

2011), which have been mentioned for decades because of their role in resting metabolic rate.

In summary, the endocrine system is likely to play an essential role in the development, ontogeny, homeostatic regulation, and evolution of many, if not most, complex traits. Although natural and sexual selection are likely to act above the level of endocrine function per se, aspects of the endocrine system are highly likely to get "dragged along" during the evolution of complex traits because they affect so many parts of the phenotypic hierarchy, from gene expression and protein synthesis to organismal performance and behavior.

The endocrine system

Endocrine communication, by definition, refers to transfer of information among different tissues or organs by chemical messengers that are transported through the blood—i.e., hormones. In addition to the diffuse collection of endocrine organs and tissues, and the hormones that they synthesize and secrete, components of the endocrine system include transport proteins, receptors, and signal transduction mechanisms (for overviews of the endocrine system, see Nelson (2011); Kronenberg et al. (2016); Lazar and Birnbaum (2016)). At the coarsest level, Shire (1976) recognized an "endocrine unit" as consisting of a stimulus generator, which provides a stimulus to an endocrine tissue, whose output is carried by a transport system and then signals target organs, which may have multiple responses. Thus, functional changes in the endocrine system, on both evolutionary and ontogenetic scales, can be mediated by changes not only in hormone secretion but also in many other components.

It is worth noting that the endocrine system is far less of a "system" than some others, such as the musculoskeletal or circulatory systems, in that it subserves myriad functions and does not necessarily operate as a coordinated unit. The evolutionary origins of the vertebrate endocrine system are diverse (e.g., see Barrington (1987); Thornton (2001); Dean and Thornton (2007); Bridgham et al. (2009); Sower et al. (2009); pages R203-R204 in Storz et al. (2015); Baker (2015); Schwartz and Bronikowski (2017)), and the ways it may respond to selection are manifold. Moreover, components of the overall endocrine system may be involved in a wide array of biological functions, such as involvement of the insulin and insulin-like signaling network in cell division and growth, organismal metabolism, growth and development, reproduction, and lifespan (Schwartz and Bronikowski 2017).

Hormones fall into two major categories, hydrophilic and hydrophobic, that differ markedly in many aspects of synthesis, secretion, transport through the blood, receptors, and inactivation/clearance from the body. Hydrophilic hormones include peptides and proteins (e.g., insulin, leptin, adiponectin, growth hormone), which make up most of the hormones in the vertebrate body. Peptide and protein hormones are produced in endocrine cells via transcription, translation, and post-translational modification, packaged in membrane-bound secretory vesicles, and released into the blood by exocytosis. Hydrophilic hormones typically circulate through the body dissolved in plasma (Nelson 2011; Kronenberg et al. 2016; Lazar and Birnbaum 2016). Because they are lipophobic, hydrophilic hormones are unable to cross plasma membranes. At target cells (i.e., cells containing specific receptors for a particular hormone), therefore, these hormones bind to membrane receptors, thereby activating signal-transduction pathways within the cell. This process can have rapid (within seconds) effects on cellular function through modification of existing proteins. In addition, signal-transduction cascades sometimes lead to changes in gene expression, a much slower process. Hydrophilic hormones are rapidly inactivated by peptidases and excreted; thus, they have short half-lives, on the order of several minutes. In addition to proteins and peptides, hydrophilic hormones include the catecholamines (epinephrine, norepinephrine, and dopamine) (Nelson 2011; Kronenberg et al. 2016; Lazar and Birnbaum 2016).

Hydrophobic hormones include steroids, thyroid hormones (thyroxine, triiodothyronine), and melatonin. Steroids (e.g., estrogen, progesterone, testosterone, cortisol, corticosterone, aldosterone) are nonpolar molecules consisting of three 6-carbon rings and a conjugated 5-carbon ring, synthesized from cholesterol by tissue-specific biosynthetic enzymes. Because they are lipid-soluble, steroid hormones diffuse readily across cell membranes and thus are secreted by parent cells immediately following synthesis. As a result of their limited solubility in plasma, they circulate in the blood bound, in large part, to carrier proteins, including albumin and hormone-specific carriers (Nelson 2011; Kronenberg et al. 2016; Lazar and Birnbaum 2016). Although the functions of these carrier proteins are not fully understood, they appear to prevent hydrophobic hormones from entering cells, thus maintaining a reservoir of these hormones in the circulation (Malisch and Breuner 2010). Only the unbound or "free" fraction is thought to be biologically active.

Unbound steroid molecules diffuse into all cells. In target cells, they bind to receptors located in either the cytoplasm or the nucleus. The hormone-receptor complex then binds to hormone-response elements in DNA and acts as a transcription factor, increasing or decreasing the expression of specific genes. These effects typically develop more slowly than those of hydrophilic hormones, requiring up to several hours, but persist longer. Some steroids can also act through membrane receptors to exert rapid, more transient effects on target cells (Gruber et al. 2002; Sakamoto et al. 2012). Steroid hormones are typically inactivated in the liver and excreted, and have half-lives on the order of hours. Thyroid hormones (thyroxine and triiodothyronine) and melatonin have mechanisms of transport and action similar to those of steroids (Kawata 2001).

Hormone secretion is regulated by neural inputs, other hormones, or specific physiological stimuli, and often involves negative feedback loops (Melmed et al. 2016). Insulin, for example, is secreted by the pancreas in response to high blood glucose concentrations and, correspondingly, acts on various tissues to lower blood glucose levels, thereby reducing its own secretion. As another example, adrenocorticotropic hormone (ACTH), a protein from the anterior pituitary, stimulates release of glucocorticoid hormones (cortisol and/or corticosterone, depending on the species) from the adrenal cortex. In turn, high concentrations of glucocorticoids feed back to the anterior pituitary to decrease secretion of ACTH. Simultaneously, ACTH secretion is stimulated by neuropeptides from the brain, corticotropin-releasing hormone and vasopressin. Thus, control of hormone release is often multifactorial. Expression of receptors, too, is dynamically regulated by such factors as concentrations of their ligands or of other hormones. Additionally, levels of many hormones, receptors, and even binding proteins may fluctuate systematically over both circadian and ultradian cycles (Malisch et al. 2008; Giguere et al. 2011; Nicolaides et al. 2014).

Interactions between hormones and behavior

Hormones can influence behavior through a multitude of actions on sensory systems, integrative processes in the central nervous system, motor output systems, and other peripheral organs and structures (for reviews, see Pfaff et al. (2004); Nelson (2011)). In many cases, a particular hormone exerts complementary effects on both central and peripheral components of a specific behavior—in other words, both the motivation and the ability to perform the behavior—and these effects can be acute and/or chronic (Nelson 2011). In female mammals, for example, the protein hormone prolactin, secreted by the anterior pituitary, both enhances the neural motivation to engage in maternal behavior and stimulates milk production in the mammary glands (Grattan 2002). As another example, testosterone, a steroid hormone secreted by the testes, acts both centrally to promote aggressiveness and libido and peripherally to develop and maintain weaponry and ornamentation (e.g., antlers, plumage coloration) used in inter-male competition and female courtship (Setchell et al. 2008; Nelson 2011; Pasch et al. 2011). This duality of function has evolved subject to the screening process of natural selection, and can be viewed as a possible example of genetic architecture and gene regulatory networks (acting through gene expression and downstream effects on phenotypes) evolving to match the prevailing selection regime (Pavličev and Cheverud 2015).

For some behaviors, endocrine regulation requires two or more hormones acting in concert. For example, estrogen and progesterone interact to stimulate the expression of sexual behavior in female rodents (Blaustein 2008); in male rodents, rhythmic diurnal changes of progesterone synergize with testosterone to facilitate sexual behavior (Witt et al. 1994; Phelps et al. 1998; Woolley et al. 2006). In some reptiles and amphibians, progesterone and testosterone synergize to promote aggression (Woolley et al. 2004; Crews and Moore 2005), whereas in other species these same hormones promote sexual behavior in males (Lindzey and Crews 1992; Lindzey and Crews 1993). As another example, regulation of appetite and food intake is coordinated by a large suite of hormones from the brain (e.g., corticotropin-releasing hormone), gastrointestinal tract (e.g., ghrelin, cholecystokinin), pancreas (e.g., insulin), adipocytes (e.g., leptin), and adrenal cortex (corticosterone or cortisol), in addition to other endocrine, neural and metabolic signals (Pfaff et al. 2004; Berthoud 2007; see also Alberts and Pickler (2012)).

Importantly, hormones can act at different life stages to influence behavioral phenotypes. According to the classic organizational-activational hypothesis (Phoenix et al. 1959), the expression of sexually dimorphic behaviors—including sexual, parental, and aggressive behaviors, among others—depends, first, on exposure to (in males) or absence of (in females) gonadal steroids (especially testosterone) at specific ''sensitive periods'' during prenatal or early postnatal development. These early-life effects more or less permanently ''organize'' the neural

substrates of the behavior. Beginning at puberty, subsequent exposure to testicular or ovarian steroid hormones transiently "activates" the expression of the behavior (Phoenix et al. 1959; Arnold 2009). Organizational and activational effects on the brain can involve changes in numbers, morphology, size, and electrophysiological properties of neurons, as well as effects on synthesis of neurotransmitters and neuropeptides, or expression of receptors for these neurocrines or hormones (Nelson 2011).

Since its original formulation in 1959 (Phoenix et al. 1959), the organizational-activational hypothesis has been refined in important ways. First, not all sex differences in behavior are organized by sex differences in early exposure to gonadal hormones; rather, some behavioral sex differences result from direct effects of genes on the sex chromosomes, from epigenetic effects, or from exposure to hormones other than gonadal steroids (Arnold 2009; McCarthy and Arnold 2011). Second, organizational effects may be less permanent than initially thought. Third, in at least some vertebrate taxa, organizational and activational effects on the male brain are not mediated directly by testosterone but rather by estrogen, which is synthesized in neurons either *de novo* or via aromatization of testosterone (Sodersten 2015). Fourth, hormonal determinants of organization and activation can differ both among and within species (Crews and Moore 2005; Adkins-Regan 2012). Finally, in addition to the classic dichotomous effects of steroids in early development and adulthood, it is now becoming clear that steroids can act during puberty to exert long-term effects on the brain and behavior (Juraska et al. 2013).

A crucial point is that hormones do not directly "cause" behavior in the sense of having a deterministic effect. Instead, hormones interact with other neural and physiological systems to influence the likelihood that particular behaviors will be expressed under certain conditions (Pfaff et al. 2004; Nelson 2011). Testosterone promotes libido in many species, for example, but high circulating concentrations of testosterone do not ensure that males will engage in sexual behavior. Instead, they increase the probability that a male will seek, court, and mate with females under conditions of appropriate organismal (e.g., low stress) and environmental (e.g., presence of an attractive female, absence of a dominant male) conditions (Nelson 2011).

Interactions between hormones and behavior can be bi-directional (Fig. 1). Returning to the examples above, not only does prolactin enhance the expression of parental behaviors in birds and mammals, but its secretion can also be increased by engagement

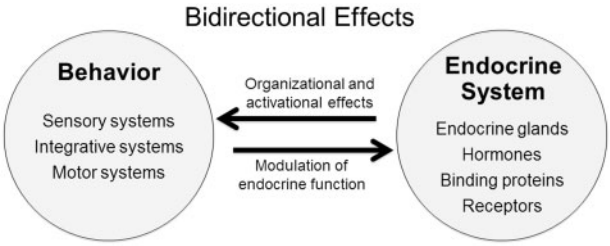

Fig. 1 Interactions between hormones and behavior can be bi-directional (see text). For example, testosterone activates both sexual and aggressive behavior in males, whereas engagement in sexual or aggressive interactions can feed back to increase secretion of testosterone.

in these behaviors and the resulting exposure to stimuli from the young (e.g., Lea et al. 1986). In a similar fashion, testosterone promotes aggressive behavior in males, whereas engagement in competitive or aggressive interactions can feed back to increase secretion of testosterone (Wingfield et al. 1990). Other examples involve negative feedback loops between hormones and behavior. For instance, high levels of leptin, a peptide hormone secreted by adipocytes, suppress appetite, leading (in theory, at least) to a reduction in food intake and, ultimately, to a decrease in leptin secretion (Zhang and Scarpace 2006; Meek et al. 2012; Balland and Cowley 2015, references therein). In general, effects of behavior on endocrine function can be mediated by numerous mechanisms, including behaviorally induced changes in metabolism, energetics, sensory input, cognition, and mood, among others.

Finally, hormonal influences on behavior may be dose- and time-dependent, and relationships between hormone levels and behavior may not be linear. Across several vertebrate taxa, for example, acute elevations of glucocorticoid hormones have permissive or stimulatory effects on aggressiveness, whereas chronic glucocorticoid elevations inhibit aggression (Summers et al. 2005). In humans, excessively high concentrations of thyroid hormones can lead to nervousness, irritability, and insomnia, whereas unusually low concentrations of the same hormones may contribute to depression and cognitive impairments (Pfaff et al. 2004). Effects of hormones on behavior, and vice versa, can also differ among species and between the sexes, can change with age, and can vary among individuals with different experiential or genetic backgrounds (Pfaff et al. 2004). Among biparental rodents, for example, testosterone inhibits the expression of paternal care by fathers in some species but enhances paternal care in others (Bales and Saltzman 2016).

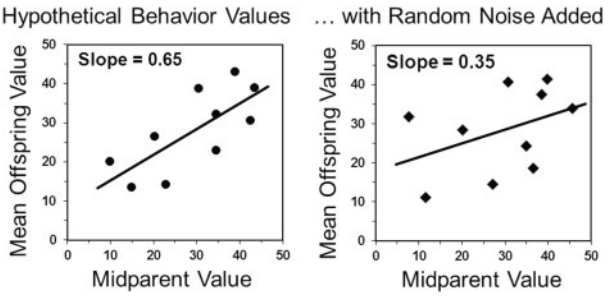

Fig. 2 Illustration of how low repeatability of behavior will reduce its narrow-sense heritability (ratio of additive genetic variance to total phenotypic variance in a population). On the left are hypothetical values for a behavioral trait for the average of the two parents and the average of their offspring (presuming that sex differences either do not exist for the trait in question or that they have been removed statistically). In this case, the estimate of the narrow-sense heritability is the slope of the least-squares linear regression (assuming no non-genetic parental effects and no shared-environmental effects). On the right, random noise has been added to the X and Y values for each of the 10 families, which results in a lowering of the strength of the relationship and hence a lower slope. A reduced tendency for a trait to "run in families" because of low repeatability (caused by high liability of the trait and/or high measurement error per se) will reduce the rate of response to directional selection, whether natural, sexual or artificial.

Selection on behavior

Behavior can be defined as what an organism does in any given situation: it is caused by motivation, but limited by ability. Most biologists who work with animals accept the general premise that selection in nature usually acts more directly on behavior than on the subordinate traits that affect behavior and determine performance abilities (e.g., see Fig. 1 in Garland and Kelly (2006), Fig. 1 in Careau and Garland (2012), Fig. 1 in Storz et al. (2015)).

Behavior differs from morphology in that it is highly labile from moment to moment and day to day. The repeatability (consistency from day to day or over other time intervals (Boake 1989)) of behavior is often relatively low, which reduces both the intensity of selection acting on behavior (behavior is a "moving target") and its narrow-sense heritability (Fig. 2). A reduced selection intensity and/or narrow-sense heritability (Stirling et al. 2002) will lower the possible rate of adaptive evolution for behavioral traits as compared with morphological traits. Of course, the same points could be made for circulating levels of many hormones (Cox et al. 2017).

Nonetheless, behavior evolves a lot and quickly, and it is often viewed as a "driver" of evolutionary change (Sol et al. 2005; but see Huey et al. (2003)), which can be termed the "Behavior Evolves First" hypothesis (Mayr 1960; Blomberg et al. 2003; Rhodes and Kawecki 2009), or at least being at the leading edge of phenotypic evolution. Darwin (1859), for example, stated that "... the acutest observer by examining the dead body of the water-ouzel would never have suspected its sub-aquatic habits. ... In such cases, and many others could be given, habits have changed without a corresponding change of structure." In any case, behavior can and does evolve rapidly, as illustrated by substantial differences among the songs of closely related species of sparrows (Nowicki et al. 2001) or among behavior of dog breeds (Draper 1995; Careau et al. 2010).

Many studies have bred rodents and other vertebrates for particular aspects of behavior (e.g., see Supplementary Table S1). For example, various livestock breeds have been bred for behavioral traits related to production farming (e.g., see Nicol (2015) on chickens). Rodents, especially laboratory house mice and laboratory Norway rats, have been common subjects of selection experiments under controlled laboratory conditions (Hyde 1981; Rhodes and Kawecki 2009; Swallow et al. 2009). House mice have been bred for a wide range of behavioral traits, including open-field behavior (DeFries et al. 1978; Flint et al. 2004; Henderson et al. 2004), thermoregulatory nesting (Lynch 1980, 1994; Bult and Lynch 2000), female agonistic behavior (from a wild-derived starting population: Ebert and Hyde (1976); Hyde and Ebert (1976); Hyde and Sawyer (1979); Hyde and Sawyer (1980)), maternal defense (Gammie et al. 2006), voluntary wheel running (Swallow et al. 1998; Rhodes and Kawecki 2009; Swallow et al. 2009), and home-cage activity (Zombeck et al. 2011; Majdak et al. 2014).

Some interesting examples from an endocrine perspective involve experimental domestication of multiple species of mammals (foxes, river otters, mink, rats) in Siberia, begun in 1959 by Belyaev. In these studies, selection for "tameness" was viewed as a first step. The response to selection in the foxes involved many correlated responses, including some at the endocrine level (Belyaev 1979; Trut 1999; Bidau and D'Elía 2009; Kukekova et al. 2012). In pups from the unselected population, at 45 days of age the fearful response to humans appears, exploratory behavior of a new environment decreases, and there is a sharp increase in glucocorticoid content in the peripheral blood. In pups from the domesticated line, at 45 days of age the fearful response does not appear, exploratory activity is not diminished, and

glucocorticoid content is not increased (Trut et al. 2004).

Domestication of rats started with 233 wild-caught *Rattus norvegicus*. They were tested for reaction to a human hand's approach in their cage. The 30% that were least aggressive were bred to form a "tame" line, while the 30% most aggressive were bred to form an "aggressive" line. No non-selected control line was maintained, so in subsequent papers regarding correlated responses it is not always easy to discern which line(s) changed. In addition, no replicate selection lines were used, so random genetic factors could account for some of the correlated responses (Henderson 1989; Henderson 1997; Koch and Britton 2005; Majdak et al. 2014; Sadowska et al. 2015).

Although the rats' responses to humans changed dramatically, conspecific aggression did not change. Several endocrine responses were observed, based on comparison of the two lines (Albert et al. 2008, and references cited therein). Serum testosterone in females was higher in the tame than in the aggressive line, although the authors downplay this result. Serum corticosterone and fecal metabolites of corticosterone were lower in the tame line, which was less reactive to restraint, pain, and injections of noradrenaline and serotonin, and had smaller adrenal glands. Brain size was larger in the tame line and brain taurine concentrations were higher, but brain serotonin and GABA concentrations were lower.

Artificial selection on endocrine function

As argued elsewhere, selection experiments can be a powerful approach for demonstrating mechanisms of behavioral or organismal-level evolution (Shire 1976; Garland 2003; Storz et al. 2015). Suppose that selection is applied to a behavioral trait, that it responds across generations, and that one or more lower-level traits are observed to change as a correlated response, consistently in replicated selection lines. One could then hypothesize that the lower-level trait (e.g., a change in the circulating levels of a particular hormone) was causally related to the change in behavior. This hypothesis could be tested with another selection experiment, directly targeting the hormone concentrations.

To our knowledge, this research approach has not yet been undertaken with any vertebrate. However, some studies have directly targeted behaviorally relevant lower-level endocrine traits. For example, laboratory mice were bidirectionally selected for iodine release rate, determined by in vivo thyroid radiation counts on the second, third, and fourth days after an injection of 5 uc I^{131} in adult mice (Blizard and Chai 1972). All else being equal (e.g., no variation in hormone receptor densities), a higher release rate is presumed to represent greater thyroid activity. A number of behavioral or behavior-relevant correlated responses were observed. Specifically, the low-release-rate line (= relatively hypothyroid) had increased defecation in an open-field test, had increased activity (and decreased latency to move) in the open field on only the first day, learned more quickly in a water-maze test, showed no difference in body, pituitary or testes mass, but had smaller adrenal glands and larger brains. Unfortunately, this selection experiment (like many others with rodents) included no control line and no replication, so it is not always clear which line(s) changed in response to selection.

More recently, replicate lines of zebra finches were bred for high or low corticosterone responsiveness to a mild stressor (20 min holding in a cloth bag), while two non-selected control lines were also maintained (Evans et al. 2006). After five generations of selection, an asymmetry in response was observed, with the high-selected lines evolving as would be expected but the low-selected lines apparently not diverging significantly from the control lines. Although circulating corticosterone concentrations differed two- to three-fold between high- and low-selected lines, no statistically significant differences were observed for circulating testosterone levels in adult males. Moreover, no consistent, significant differences were found for any of the life-history traits studied. A subsequent report of generations 4-5 indicated an increase in body size for birds in the low-corticosterone lines, but little effect on male sexual signal quality or dominance ranking, and no simple relationship with measures of immune function (Roberts et al. 2007).

With the wide availability of ELISAs for various hormones, we see excellent prospects for additional studies that selectively breed for endocrine function and then study correlated responses in behavior, life history, etc.

Selective breeding for wheel running

As emphasized elsewhere (Henderson 1989; Henderson 1997; Garland and Rose 2009; Rhodes and Kawecki 2009; Kawecki et al. 2012), replication and control lines are crucial for strong inference with selection experiments. Therefore, the selection experiment that our laboratory began in 1993 includes four replicate lines bred for high levels of voluntary

wheel-running behavior (High Runner [HR] lines) and four non-selected control lines (Garland 2003; Swallow et al. 2009). (It does not include any lines selected for low wheel running: we did not intentionally create "couch potatoes.") The starting (base) population was 112 male and 112 female mice of the outbred Hsd:ICR strain (Swallow et al. 1998). This strain exhibits levels of genetic variation similar to populations of wild house mice and to human populations, and has been used in other selection experiments and several quantitative-genetic analyses of various traits (e.g., Dohm et al. (2001)).

The selection criterion is the total number of revolutions run on days 5 and 6 of a 6-day period of wheel access when mice are young adults. We chose to select after a few days of familiarization with the wheels, which are attached to standard housing cages, to avoid selecting on neophobia (Kronenberger and Medioni 1985). Wheels are large (1.12 m in circumference, the size typically used for rats), reflecting an experimental design choice that anticipated possible future studies of wheel running in other species of rodents (Dewsbury 1980; Chappell and Dlugosz 2009). Further details of the selection protocols are provided elsewhere (Swallow et al. 1998; Garland 2003; Swallow et al. 2009; Careau et al. 2013).

At the outset, we had some very general hypotheses and predictions (see also Rhodes et al. (2005)). First, we expected that selective breeding for high voluntary wheel running would lead to changes in both motivation and ability. Hence, subordinate (lower-level) traits that evolved would involve both neurobiology and exercise physiology. This crudely separates into brain vs. body, so we expected that the structural genes that directly affect motivation vs. ability would be largely different. However, we also presumed that upstream regulatory control of gene expression could share some pathways.

Moreover, certain mechanisms "used" by selection mainly because they affect motivation or ability (e.g., changes in circulating levels of, or receptors for, a particular hormone) might affect numerous other traits, within or between these domains, leading to numerous correlated responses, some of which might be only tangentially related to wheel running. Some of the lower-level traits that evolved might even be somewhat detrimental to aspects of wheel running, other behaviors or reproduction.

In other words, within the general domains of motivation or ability, many of the genes whose frequencies change would likely have pleiotropic effects on other aspects of behavior or physiology. These effects might or might not be predictable. Although we do know a lot about "how organisms work," the pathways from genes to even lower-level traits (the so-called genotype-phenotype map) are not well understood.

In addition to those general hypotheses, we also predicted changes in a number of exercise-related traits in the HR lines, including increases in endurance capacity, maximal oxygen consumption, blood hemoglobin concentration (or hematocrit), relative heart size, and changes in muscle characteristics. As outlined below, most of these predictions have turned out to be accurate.

Mice in the high-selected lines diverged rapidly from controls (Swallow et al. 1998) and reached apparent selection limits at generations 16–28, depending on sex and line (Careau et al. 2013). The gradual increase in running behavior over many generations is consistent with polygenic inheritance, as would be expected for such a high-level, complex trait. Running on days 1–4 has also increased in the HR lines, with some important changes in the additive-genetic variance-covariance matrix for running across the days (Careau et al. 2015). (An analysis of an advanced intercross population involving one of the four HR lines demonstrated some separate quantitative trait loci affecting days 1–4 versus 5–6 (Kelly et al. 2011).) Interestingly, the increase in distance run was caused mainly by an increase in average running speed, although males also show an increase in running duration (Garland et al. 2011a). Although the HR lines evolved to be smaller in body size and to have reduced body fat (Girard et al. 2007), differences in life-history traits (weaning success, litter size, total litter mass, mean offspring mass, sex ratio at weaning) had not evolved as of generations 21–22 (Girard et al. 2002).

As of the current generation (76), various aspects of exercise physiology and morphology have changed in the HR lines, including increases in endurance capacity (Meek et al. 2009), maximal oxygen consumption (Dlugosz et al. 2009; Kolb et al. 2010), heart size (Kolb et al. 2010), size of femoral head and hindlimb symmetry (Garland and Freeman 2005), along with reductions in muscle mass (Garland et al. 2002; Kelly et al. 2013) and alterations in muscle fiber type (Bilodeau et al. 2009). In addition to an increased ability for aerobically supported exercise, mice from the HR lines have alterations in the brain reward system that presumably indicate higher motivation for wheel running (Rhodes et al. 2005; Belke and Garland 2007; Rhodes and Kawecki 2009; Keeney et al. 2012). Moreover, the HR lines exhibit some evidence of being addicted to wheel running (Kolb et al. 2013).

Although the HR lines have evolved to be smaller in both body mass and length under most conditions (Swallow et al. 1999; Kelly et al. 2006; Copes et al. 2015), it is not entirely clear if this would be beneficial for sustained, endurance-type exercise. A similar question concerns their generally reduced body fat (Swallow et al. 2001; Girard et al. 2007; Nehrenberg et al. 2009; but see Vaanholt et al. (2007b); Meek et al. (2014); Acosta et al. (2015)). Studies of circulating hormones (next section) indicate that two produced by white adipose tissue (adiponectin, leptin) show altered concentrations in the HR mice, even after adjusting statistically for body fat.

The HR lines show evidence of increased adaptive plasticity for some traits (Houle-Leroy et al. 2000; Garland and Kelly 2006; Kelly et al. 2006; Middleton et al. 2008). Perhaps most dramatically, HR and C mice did not differ significantly in gastrocnemius GLUT-4 transporter protein abundance when housed without wheels, but after five days of wheel access HR mice had 2.4-fold more GLUT-4 than C mice (Gomes et al. 2009). This difference was not a simple linear function of the amount of wheel running on the previous day, which is much higher in HR mice. Therefore, this is arguably a case of the evolution of increased ''self-induced adaptive plasticity'' in the HR lines (Swallow et al. 2005).

Endocrine studies of the HR mice

The potential bidirectional relationships (Fig. 1) between endocrine function and exercise behavior are manifold (e.g., Radosevich et al. 1988; Coleman et al. 1998; Girard and Garland 2002; Droste et al. 2007; Sinclair et al. 2014). Accordingly, a number of studies have addressed aspects of endocrine differences between HR and control mice, with the vast majority considering circulating hormone concentrations (adiponectin, corticosterone, IGF-1, insulin, leptin, testosterone, thyroxine, triiodothyronine). A few studies have reported masses of endocrine organs (adrenal glands, ovaries, testes: Klomberg et al. 2002; Swallow et al. 2005; Malisch et al. 2007). In total, these studies are far from providing a complete picture of endocrine evolution in the HR lines, in part because they have mostly included only one sex, age, generation, and housing condition (e.g., with or without wheels). The following paragraphs highlight some of the findings. A few studies with collaborators in the Netherlands have, due to logistical constraints, included only a subset of the HR and control lines, and these will not be considered here (Vaanholt et al. 2007a; Vaanholt et al. 2008; Guidotti et al. 2016).

Mice from the HR lines have higher baseline adiponectin levels, even after accounting statistically for their lower body fat (Vaanholt et al. 2007b). Adiponectin is a protein hormone produced by adipose tissue and is the most abundant adipokine in the circulation that can access the brain. It has many functions, with more being discovered on a regular basis (Stofkova 2009; Galic et al. 2010). Adiponectin is involved in energy balance, glucose regulation, lipolysis, and some aspects of the brain reward system. In general, it is viewed as having a positive role in metabolic health, working against the metabolic syndrome and type 2 diabetes. Circulating concentrations of adiponectin are also known to respond to physical exercise (Saunders et al. 2012). Therefore, it seems possible that this hormone is involved in both motivation and ability of the HR mice.

Baseline plasma corticosterone concentrations are elevated approximately two-fold in HR mice of both sexes (Malisch et al. 2007). We have found no differences in the circadian pattern of blood corticosterone concentrations nor in levels of corticosterone-binding globulin, and so we believe that the higher corticosterone levels likely have functional significance (Malisch et al. 2008). Glucocorticoids are not just ''stress hormones'' in the conventional sense. They are generally involved in energy mobilization during exercise, may stimulate physical activity (Sibold et al. 2011; Malisch et al. in revision; references therein), and may interact with the brain's reward system. Various studies of multiple species of mammals, including the HR mice, show that circulating corticosterone levels respond to physical exercise, both acutely and chronically (Coleman et al. 1998; Girard and Garland 2002; Droste et al. 2007). As with adiponectin, it seems that this hormone may be involved with both motivation and ability of the HR mice.

Although one can make a case that elevated corticosterone levels have evolved adaptively in the HR lines, we have also considered the possibility that they could be a nonadaptive or even maladaptive byproduct of changes in the brain (e.g., the hypothalamus: Malisch et al. 2007). Elevated glucocorticoids can reduce growth rates in mammals, and we have found correlational evidence that this may have occurred in the HR mice. Specifically, mean baseline corticosterone levels for each sex are negatively related to body mass across the eight lines (Malisch et al. 2007). In addition, at the level of individual variation, growth over days 26–55 of age was negatively related to mean nighttime corticosterone levels in females from both HR and control lines (Girard and Garland 2002). Most recently, we have found that adding corticosterone hemisuccinate to drinking

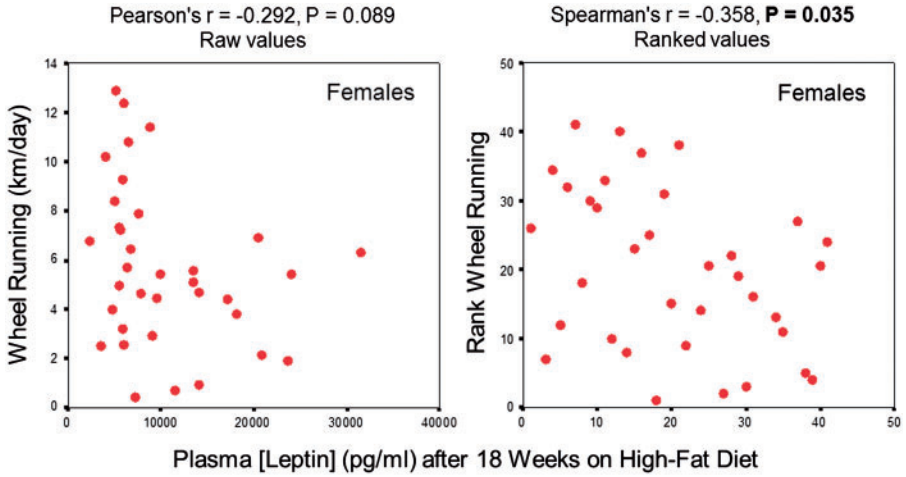

Fig. 3 Among 35 inbred strains of mice, mean daily wheel-running distance is negatively related to circulating leptin levels in females, but not for males (values not shown). The relationship is statistically significant for ranked values. Data are from the Mouse Phenome Database at The Jackson Laboratory (http://phenome.jax.org/). Values for wheel running are from Lightfoot (http://phenome.jax.org/db/q?rtn=projects/details&sym=Lightfoot1), whereas leptin levels are from Naggert (http://phenome.jax.org/db/qp?rtn=views/measplot&brieflook=14306&projhint=Naggert1).

water confirms the negative effects on growth rates from weaning to adulthood in males of both HR and control lines (J. S. Singleton and T. Garland, unpublished results).

Much as glucocorticoids have been pigeon-holed as "stress hormones," leptin has perhaps been overemphasized as the "obesity hormone." In fact, the endocrine effects of leptin in both peripheral tissues and the central nervous system are manifold (e.g., Zhang and Scarpace 2006; Stofkova 2009; Galic et al. 2010; Balland and Cowley 2015; Houweling et al. 2015) and should be viewed from a broader perspective. Relatively few studies have examined leptin's effect on voluntary exercise (e.g., Choi et al. 2008; Morton et al. 2010; Meek et al. 2012; Fernandes et al. 2015); however, given its pervasive effects, it is safe to presume that leptin may influence both motivation and ability for voluntary exercise.

Consistent with their usually lower amounts of body fat, young adult female HR mice have lower baseline circulating leptin levels than do mice from the control lines. This difference is statistically significant even when using body fat as a covariate in the statistical model (Girard et al. 2007). However, not all studies have found reduced leptin levels in HR mice (Vaanholt et al. 2007b), and one report indicates dramatically different effects of early-life wheel access on adult leptin levels in the HR (decreased) and their control (increased) lines (Acosta et al. 2015).

The Mouse Phenome Database (MPD; Jackson Labs) provides a wealth of tools for exploring both phenotypic and genetic associations of traits in laboratory strains of house mice (Bult 2012), which now include some wild-derived strains of other species. Perusal of this data base will make clear that mouse strains show tremendous diversity in many aspects of behavior and endocrine function (e.g., see Spearow et al. (1999); Svenson et al. (2007); Li et al. (2008); Miller et al. (2010)). We searched the MPD for information on wheel running and hormone levels for inbred strains. Among 35 inbred strains of mice, mean daily wheel-running distance is negatively related to circulating leptin levels (Svenson et al. 2007) in females (Fig. 3) but not in males (data not shown). The relationship is statistically significant for ranked values. Further support for a negative relationship between circulating leptin levels and physical activity is provided by various studies of humans (e.g., Franks et al. 2003; Holtkamp et al. 2003: see also Girard et al. (2007)). An important area for future research will be determining whether maternal variation in circulating hormone levels is affecting adult phenotypes of their offspring, possibly in a way that contributes to the selection limits observed in all four of the HR lines (Careau et al. 2013; Careau et al. 2015; Garland et al. 2017).

Conclusions and future directions

As demonstrated by the examples cited above, complex traits, such as most behaviors, evolve in complicated ways. Artificial selection on vertebrate behavior commonly results in correlated endocrine responses. However, few studies have demonstrated

that the endocrine changes are causally related to the evolutionary changes in behavior. Subsequent studies selecting on endocrine function might provide compelling evidence for causality (Garland 2003), but this has yet to be attempted. Nevertheless, the endocrine system includes so many "moving parts" (e.g., see Shire (1976); Young and Crews (1995)) that it may often respond in diverse ways to selection at the level of behavior: "multiple solutions" (Turner et al. 2010; Losos 2011; Garland et al. 2011a) seem likely to be the rule, rather than the exception.

Endocrine-level correlated responses to selection on behavior may occur for several reasons. First, they may represent mechanisms underlying behavioral change at the levels of motivation and/or ability. In this capacity, these effects could occur early in life (i.e., organizational effects), possibly through epigenetic mechanisms, and/or later in life (activational effects). More generally, hormones have the potential to mediate suites of traits that either facilitate or constrain adaptation (or responses to sexual selection), depending on the details of multivariate selection acting on those traits (McGlothlin and Ketterson 2008; Pavličev and Cheverud 2015; see also Garland (1994)).

Second, endocrine responses to selection on behavior may represent ontogenetic or immediate effects of the altered behavior, such as acute changes in circulating corticosterone levels in response to physical activity or longer-term changes resulting from exercise training over many days or weeks (e.g., Droste et al. 2007). As an example of the former, HR mice have higher corticosterone levels compared to control mice during the scotophase, as an acute response to their increased running (Girard and Garland 2002). (The HR mice also have higher baseline circulating corticosterone levels than controls when sampled at rest, during the day (Malisch et al. 2007).)

Third, pleiotropic gene action (reflecting functional relationships) is expected to be pervasive for many aspects of the endocrine system, such that various components may get "dragged along" by selection on higher-level traits. As a consequence, some of the endocrine changes that are observed in response to selection on a particular behavior (e.g., voluntary wheel running) might be nonadaptive or even maladaptive "byproducts" of selection on the target behavior.

Although the approach of selection experiments and experimental evolution (Garland and Rose 2009) is an excellent way to study the consequences of selection on behavior (Garland 2003; Rhodes and Kawecki 2009), establishing causal evolutionary relationships between hormones and behavior remains a difficult undertaking. Nonetheless, the endocrine system is well positioned to mediate coordinated changes in multiple levels of subordinate traits underlying behavior, given the pervasive effects of hormones on sensory systems, integrative systems, motor systems, and effectors. In many cases, these effects should be general enough as to apply to vertebrates in the wild, not just the animals and conditions typically used for selection experiments (e.g., see Supplementary Table S1).

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Supplementary data

Supplementary data available at *ICB* online.

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