

Neurobiology of Mice Selected for High Voluntary Wheel-running Activity¹

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SYNOPSIS. Selective breeding of house mice has been used to study the evolution of locomotor behavior. Our model consists of 4 replicate lines selectively bred for high voluntary wheel running (High-Runner) and 4 bred randomly (Control). The major changes in High-Runner lines appear to have taken place in the brain rather than in capacities for exercise. Their neurobiological profile resembles features of human Attention Deficit Hyperactivity Disorder (ADHD) and is also consistent with high motivation for exercise as a natural reward. Both ADHD and motivation for natural rewards (such as food and sex), as well as drugs of abuse, have been associated with alterations in function of the neuromodulator dopamine, and High-Runner mice respond differently to dopamine drugs. In particular, drugs that block the dopamine transporter protein (such as Ritalin and cocaine) reduce the high-intensity running of High-Runner mice but have little effect on Control mice. In preliminary studies of mice exercised on a treadmill, brain dopamine concentrations did not differ, suggesting that changes in the dopamine system may have occurred downstream of dopamine production (*e.g.*, receptor expression or transduction). Brain imaging by immunohistochemical detection of *c-Fos* identified several key regions (prefrontal cortex, nucleus accumbens, caudate-putamen, lateral hypothalamus) that appear to play a role in the differential response to Ritalin and in the increased motivation for running in High-Runner mice. The activation of other brain regions, such as the hippocampus, was closely associated with wheel running itself. Chronic wheel running (several weeks) also increased the production of new neurons to apparently maximal levels in the hippocampus, but impaired learning in High-Runner mice. We discuss the biomedical implications of these findings.

INTRODUCTION

Neurobiology aims to understand the underlying physiological, biochemical, and molecular basis of behavior. In the U.S., neuroscience research is funded primarily by the National Institutes of Health (NIH). Thus, research objectives tend to focus on abnormal human behaviors and mental disorders. Nonetheless, use of “animal models” to elucidate both “normal” and “abnormal” human behavior is common (Nestler *et al.*, 2002; Carroll *et al.*, 2003). Because many mental disorders are influenced by genes (*e.g.*, Attention Deficit Hyperactivity Disorder [Solanto *et al.*, 2001], schizophrenia [Kallmann, 1994], alcoholism [Enoch and Goldman, 2001]), the aim is often to find an animal model that reflects the genetic component. Currently, the most common approach within neuroscience is to use genetic engineering (Rhodes and Crabbe, 2003). Candidate genes, hypothesized to play a role in the etiology of a mental disorder, are altered and the behavioral consequences are studied (*e.g.*, Gaietdinov *et al.*, 1999). One disadvantage of this approach is that currently only one or two genes can be manipulated at a time, whereas most behaviors are influenced by many genes, interacting with each other and with numerous environmental factors. Thus, single- or double-gene models often lack face validity

(Katz and Higgins, 2003). In addition, with genetic engineering, the specific alteration that is introduced into the genome might not occur naturally, and therefore might not be relevant to the mental disorder it is intended to model. Another disadvantage of genetic engineering is that the investigator has no precise way of predicting what effect the gene manipulation will have on a particular behavior, and so animal models of specific mental disorders are often discovered by accident (*e.g.*, Nelson *et al.*, 1995).

Selective breeding provides a powerful alternative to genetic engineering because it allows for the possibility that multiple genes will contribute to the divergent phenotype (*i.e.*, the animal model) (Falconer and Mackay, 1996). Moreover, selective breeding acts on pre-existing genetic variation, and thus the resulting animal model represents a variant of nature that displays an extreme form of the behavior relative to the population at large (*i.e.*, analogous to a mental disorder). Finally, with selective breeding, a behavior itself can be directly manipulated in the direction of interest so that specific symptoms of mental disorders can be achieved by design (if they are embodied within the selection criterion) without having to wait for fortuitous outcomes.

The aim of this paper is to describe a case study of how a selective breeding experiment for increased voluntary wheel-running behavior in house mice (Swallow *et al.*, 1998a) was used to investigate the neural basis of genetic “hyperactivity” (Rhodes *et al.*, 2003a). From a biomedical perspective, genetic hyperactivity is relevant as a symptom of Attention Def-

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icit Hyperactivity Disorder (ADHD) and possibly as a model of addiction to exercise as a natural reward. From an evolutionary perspective, this experiment has general heuristic value because the original goal, as stated in the original National Science Foundation proposal (funded by the Animal Behavior Panel in 1991), was to “elucidate the genetic and physiological bases of voluntary wheel-running behavior and simultaneously to study the correlated evolution of behavior and physiology.” It was hypothesized that a genetic response to selection for high activity levels would, at some point, be accompanied by increases in underlying exercise physiological capacities, and that responses at the morphological or exercise-physiological level would occur *after* significant increases in wheel running had occurred. It was argued that this experiment would constitute a direct test of the long-standing idea that behavior evolves more rapidly than does morphology or exercise physiology (*e.g.*, see references in Blomberg *et al.*, 2003; Huey *et al.*, 2003).

As detailed elsewhere (Swallow *et al.*, 1998a; Swallow *et al.*, 2005), we maintain 4 replicate High-Runner lines and 4 replicate Control lines, and we always test the effect of selection relative to the variation among lines (*i.e.*, degrees of freedom relate to the number of lines not individuals). The mice in our model are each housed individually with access to a running wheel (starting at age of about 40–50 days) for 6 days and the total revolutions on days 5 and 6 are used as the selection criterion to choose breeders. When mice of this age are given access to wheels, their running distance typically increases each day (*e.g.*, see Swallow *et al.*, 2001; Belter *et al.*, 2004). If we were to leave mice on the wheels for more than 6 days, the level of running would continue to increase and would stabilize only after approximately 3 weeks. Thus, given this selection protocol, it is possible that the High-Runner mice may have evolved to show a more rapid increase in wheel running after initial exposure, though we have not yet studied this in detail. We decided to allow the mice 6 days of running because we were concerned that activity on the first day or two might reflect primarily the response to novelty (*i.e.*, the novel experience of having a wheel), and we were not interested in this but rather the expression of locomotor behavior *per se*. Allowing more time with wheels would have lengthened the selection protocol and hence generation time, and also increased the possibility of training effects (phenotypic plasticity: see Swallow *et al.*, 2005).

Our original interest was mainly to explore how exercise physiological traits would evolve in a correlated fashion to support the increased activity levels. Such traits as maximum oxygen consumption, heart mass, and muscle mass were monitored across generations (Garland, 2003). Subsequently, research was initiated to explore the central nervous system with the idea that specific changes might have taken place to increase motivation for physical activity (Rhodes and Garland, 2003; Rhodes *et al.*, 2003a). Thus, the experiment demonstrates the power of selective breeding

as a tool in integrative biology, one that can be valuable across a wide range of disciplines, and one that can lead to unforeseen applications and discovery.

BEHAVIORAL PROFILE OF THE HIGH-RUNNER MICE

The selective breeding was dramatically successful. At generation 10, when the first report was published, High-Runner mice ran, on average, 75% more revolutions per day as compared with Controls (Swallow *et al.*, 1998a); by generation 16, the differential was approximately 2.7-fold, and it remained approximately at this level through generation 31 (Garland, 2003; unpublished results). In principle, the selected lines could have achieved increased revolutions per day by running more often and/or faster. As it turns out, the High-Runner mice accomplish the greater distance primarily by running faster rather than by increasing the amount of time spent running, especially for females (Girard *et al.*, 2001; Koteja and Garland, 2001). Interesting differences among the replicate lines also exist (unpublished results).

When housed on a 12:12 photo period, the 24-hour pattern of wheel running is similar between High-Runner and Control mice. Shortly after the lights go off, mice of both line-types begin to run and they continue to run for the majority of the dark period (see Fig. 1 in Girard *et al.*, 2001; Fig. 1 in Girard and Garland, 2002). Wheel running is highly intermittent in both line-types, but High-Runner females videotaped during 5 minutes of peak running exhibited shorter bouts as compared with Controls (10 *vs.* 17 sec, respectively), they ran faster during those bouts (40.8 *vs.* 20.4 revs/min), the bouts occurred more frequently (7.8 *vs.* 3.4 bouts/min), they paused for less time between bouts (2.7 *vs.* 7.4 sec), and they exited the wheel less frequently (0.4 *vs.* 0.8 exits/min) (Girard *et al.*, 2001). When tested in constant darkness or in constant light, High-Runner mice show a free-running circadian period that is shorter by about 0.5 hours (Koteja *et al.*, 2003). Moreover, the difference remained statistically significant even when the effects of time spent running and running speed were controlled statistically, so something more fundamental than just duration or intensity of wheel running must underlie the difference.

The High-Runner mice appear to be generally more active than Controls when tested in a home cage to which they have acclimated, using a method that captures intensity or speed (as opposed to duration) of activity. For example, female High-Runners were 3.9-times as active as Control mice during the second 24-hour period of housing without wheels in rat-size cages, using total number of consecutive photo-beam breaks as the measure of activity (“Ambulations” in Rhodes *et al.*, 2001). This measure is proportional to the distance traveled, or average speed of horizontal movement over 24 hours (San Diego Instruments; <http://www.sd-inst.com/prod.cagerackphoto.htm>).

In a separate study, animals were observed in their home cages after several weeks of acclimation with continuous access to wheels that were either locked to

prevent rotation or free to rotate. Focal animal observations were conducted with instantaneous sampling in which every 10 seconds the observer checked 1 or 2 of a list of 27 possible behaviors, falling under such categories as Cage Locomotion, Running, Climbing, Drinking, Eating, and Sleeping, to estimate percentage time spent engaged in the different activities (Koteja *et al.*, 1999). Note that with regard to locomotor activities (Cage Locomotion, Running, Climbing), the method provides a relative measure of duration or frequency of behavior, but does not give speed or intensity at which it is conducted. The percent time spent engaged in Cage Locomotion did not differ between High-Runner and Control mice (Koteja *et al.*, 1999), suggesting that High-Runner mice do not move more often in their cages than Controls. Taken together with the photobeam results, these findings suggest that the High-Runner mice move more quickly but not necessarily more often in their cages, which parallels results for wheel running (see above). It should be noted that in the Koteja *et al.* (1999) study, very little time (approximately 5%) was spent engaging in Cage Locomotion in any group (High-Runner or Control with free or locked wheels), giving low power to detect differences if small differences were to exist. The vast majority of time (approximately 70%) was spent in the wheels, and female High-Runner mice spent a greater amount of time climbing in their locked wheels than controls—apparently, trying to run (Koteja *et al.*, 1999). Thus, the High-Runner mice appear to be generally more active than Controls after acclimating to their environment, and this difference is most visible when the method is sensitive to changes in intensity or speed of locomotion rather than duration (though smaller—and therefore harder to detect—changes in duration and frequency are also likely to have occurred as was demonstrated in Koteja *et al.*, 1999 for climbing in the wheels).

The increase in speed or intensity of activity seen in the High-Runner mice does not extend to tests of activity in a novel environment. For example, High-Runner mice are not more active than Controls in a 3-min open-field test, which is considered a measure of exploratory behavior or reaction to a novel environment (Bronikowski *et al.*, 2001). Taken as a whole, these results are important because they suggest that different genes control activity in a novel *vs.* familiar environment. Moreover, they are consistent with a selection experiment in which lines of mice bred for increased activity in a novel open-field test showed no increase in voluntary wheel running (DeFries *et al.*, 1970).

Another interesting behavioral difference is that High-Runner mice build smaller thermoregulatory nests than Controls (Carter *et al.*, 2000). The conclusion that these seemingly disparate behaviors are jointly affected by some of the same genes is supported by results of another complementary selection experiment in which mice bred to build smaller nests displayed

increased wheel running as compared with their control lines (Bult *et al.*, 1993).

High-Runner mice also display increased predatory aggression towards crickets as compared with Control mice. However, they apparently do not differ with respect to either inter-male aggression or maternal aggression in defense of pups (Gammie *et al.*, 2003).

NEUROBIOLOGY OF HYPERACTIVE WHEEL-RUNNING BEHAVIOR

In our model, substantial individual variation in voluntary wheel running existed in the base population and still exists within both High-Runner and Control lines (Swallow *et al.*, 1998a; Garland, 2003). Individual variation (with some additive genetic basis) is, of course, required for selective breeding, but, it begs the question: what is the basis of all this variation? One possibility is that it reflects differences in traits that determine exercise capacity. In other words, what differentiates high runners from other individuals is that they have the biochemistry, physiology, and morphology that enables them to run farther (Garland, 2003). If this were the case, then we would expect selection to produce large changes in traits that determine exercise capacity, such as $\dot{V}O_2\max$, the maximum rate at which oxygen can be consumed.

An alternative possibility is that mice, in general, choose not to run at or even near the limits of their exercise capacity. Instead, what differentiates high-running individuals is not their capacity for exercise but their motivation to run (Rhodes *et al.*, 2003a). If this were the case, then we would expect the major changes to occur in the brains of High-Runner mice rather than in their muscles, hearts or lungs. As it turns out, relatively few changes in exercise-related traits have occurred in the High-Runner mice and none seem able to explain the large differential in wheel running between High-Runner and Control mice (Garland, 2003; Rhodes *et al.*, 2003a; Swallow *et al.*, 2005). Of particular interest is $\dot{V}O_2\max$. Mass-adjusted values are slightly higher in the High-Runner lines as a group (*e.g.*, 6% in Swallow *et al.*, 1998b), but the difference has only been statistically significant ($P < 0.05$) in that one study, whereas three others (at later generations) have yielded non-significant differences (unpublished data). Had our selection criterion been based on forced exercise, we might have seen a greater change in $\dot{V}O_2\max$, as was found in rats that were selectively bred for running distance to exhaustion in a forced treadmill exercise routine (Henderson *et al.*, 2002). However, our selection was conducted on a voluntary behavior, and as such, it is perhaps not surprising that the major changes appear to have taken place in the central nervous system (CNS). We now discuss several hypotheses regarding the CNS basis for high wheel running in the selected lines.

Circadian brain circuitry

One of the first studies initiated to investigate possible neurobiological differences between the High-

Runner and Control mice was motivated by findings from another selection experiment, the one on thermoregulatory nest-building behavior that was mentioned above (Bult *et al.*, 1993). In the nest-building selection experiment, the smaller nesters exhibited both elevated wheel running and a larger number of arginine vasopressin (AVP) neurons in the suprachiasmatic nucleus (SCN) as compared with the high nesters and the control lines (Bult *et al.*, 1992). Therefore, Swallow *et al.* (1998a) hypothesized that our High-Runners would display a larger number of AVP neurons in the SCN as compared with Controls, as well as build smaller thermoregulatory nests. The hypothesized difference in nest building was indeed observed (Carter *et al.*, 2000). However, no differences in number of AVP neurons were found between High-Runner and Control mice (Hochstetler *et al.*, 2004). The SCN has long been implicated in the circadian rhythmicity of wheel running, and the High-Runner mice display a shorter free-running circadian period than Control mice under both constant light and constant dark (Koteja *et al.*, 2003). Thus, some aspect of the SCN, if not AVP neurons, is likely to have changed in the High-Runner mice to account for the difference in free running periods (Koteja *et al.*, 2003). This will be a topic of future investigation.

The dopamine hypothesis

A second line of neurobiological research was initiated to explore the hypothesis that motivation for wheel running had evolved in the High-Runner lines. A literature review indicated that little was known about the neural basis of motivation for wheel running (Sherwin, 1998), except that it might act as a natural reward (Belke, 1996; Werme *et al.*, 2002; Eikelboom and Lattanzio, 2003) and therefore display features in common with other rewarding behaviors, such as eating (Kelley and Berridge, 2002) or taking drugs of abuse (Nestler *et al.*, 2001), for which a vast literature exists. If mice derived some pleasure from wheel running, then that would explain why they run in the first place, because wheel running is not a goal-oriented behavior (Sherwin, 1998). We also consulted literature on the etiology of ADHD because human subjects with ADHD display prominent symptoms of hyperactivity that are largely genetically determined (Solanto *et al.*, 2001). We used this information to help us develop hypotheses regarding which neural substrates were most likely to be involved in the increased wheel running of High-Runner mice. Interestingly, research from both the motivation/natural reward and ADHD angles pointed to the function of one particular neurotransmitter in the brain: dopamine (Berridge and Robinson, 1998; Solanto *et al.*, 2001). Dopamine was also suspect because of its role in voluntary movement (Freed and Yamamoto, 1985; Drago *et al.*, 1994; Baik *et al.*, 1995; Brudzynski and Gibson, 1997) and because it has been associated with predatory aggression (Jimerson and Reis, 1973; Schmidt, 1979, 1983; Baggio and Ferrari, 1980; Siegel *et al.*, 1999), which is

elevated in the High-Runner mice (Gammie *et al.*, 2003).

The hypothesis that High-Runner mice display altered dopamine function was first approached via behavioral pharmacology, but could have been tested using other techniques. For example, we could have compared dopamine release in High-Runner *versus* Control mice through use of brain microdialysis (Damsma *et al.*, 1992; Castner *et al.*, 1993; Hattori *et al.*, 1994; Paulson and Robinson, 1994; Salamone, 1996; Becker *et al.*, 2001) or we could have compared the anatomy of dopamine neurons by histology (Ross *et al.*, 1976; Baker *et al.*, 1980; Fink and Reis, 1981; Zaborszky and Vadasz, 2001). However, both of these approaches are quite time consuming and focus on specific mechanisms of altered dopamine function (*e.g.*, altered dopamine release or number of dopamine neurons). In addition, microdialysis is technically challenging for a mouse-sized brain, although it is commonly done (*e.g.*, Kehr *et al.*, 2001). Thus, we chose to start with pharmacology because it gives quick results and can identify differences in dopamine function that might arise from many different types of mechanisms. For example, behavioral pharmacology might yield positive results if High-Runner mice differed from Controls in: dopamine production, release, degradation or clearance, number of dopamine neurons, distribution or expression of dopamine receptor subtypes throughout the brain, or composition of the second messenger systems that comprise the full dopamine signaling pathway (Rhodes and Garland, 2003).

In behavioral pharmacology, animals are administered a drug, and its effect on one or more behaviors is monitored by comparison with animals that are administered only the vehicle (*e.g.*, saline) (Iversen and Iversen, 1975). We hypothesized that High-Runner mice would respond differently than Controls to dopamine drugs because of specific (but then unknown) alterations in the dopamine system. This prediction has been strongly supported (Rhodes *et al.*, 2001; Rhodes and Garland, 2003).

The list of dopamine drugs tested to date includes methylphenidate (Ritalin), cocaine, apomorphine, GBR 12909, SCH 23390, and raclopride. Each of these drugs interacts with the dopamine system in a different way and with varying degrees of specificity. Some of the most interesting results occurred for the drugs classified as “dopamine transporter blockers” (Ritalin, cocaine, GBR 12909). At the cellular level, all of these drugs prevent a protein called the “dopamine transporter” (DAT) from conducting its normal function, which is to transport dopamine from outside the neuron back into the neuron (Chen and Reith, 2000). Thus, when dopamine is released, during normal brain function, and DAT is blocked, dopamine is able to interact with receptors on the exterior surface of cells in the brain for a longer period of time than normal. Ritalin and cocaine also block similar transporter proteins of other neurotransmitter systems, such as norepinephrine and serotonin (Li *et*

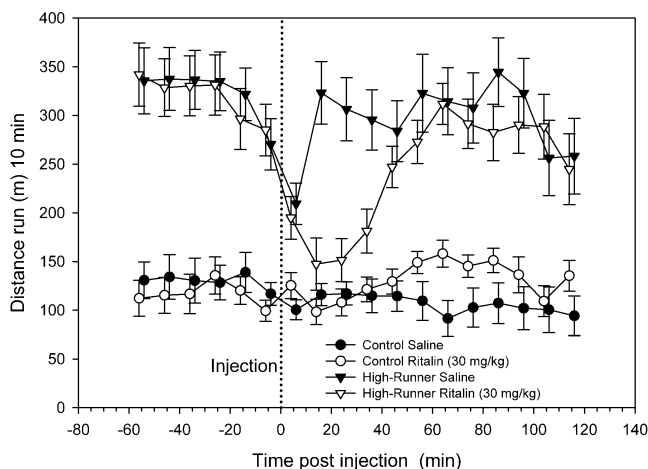


FIG. 1. Ritalin causes a large decrease in wheel running in High-Runner mice but slightly elevates running in Control mice. Mean wheel running (meters) is plotted in 10-min increments 1 hour before and 2 hours after an injection of either saline or Ritalin (30 mg/kg). High-Runner and Control mice are plotted separately. Data points are plotted at the mid-point of the interval (*i.e.*, 5 on the x-axis represents the 0–10 min interval post-injection). Modified from Figure 1 of Rhodes and Garland (2003).

al., 1996; Kuczenski and Segal, 1997). However, GBR 12909 has a high degree of specificity for DAT (Matecka *et al.*, 1996). GBR 12909 is an interesting drug because it is currently undergoing clinical trials for use as a substitution therapy for cocaine abuse (analogous to methadone treatment for heroin addicts) (see URL: <http://www.clinicaltrials.gov/ct/show/NCT00051896?order=10>).

Over a wide range of doses, all three of the DAT blockers (Ritalin, cocaine, GBR 12909) had either no effect or increased wheel running in Control females, whereas they produced a large decrease in wheel running in High-Runner mice (*e.g.*, see Ritalin response in Fig. 1). Most interestingly, the decrease in running that occurred in High-Runner mice in response to the DAT blockers resulted from a decrease in the speed of running rather than a change in the duration of running (Rhodes *et al.*, 2001; Rhodes and Garland, 2003). This is important because recall that increased speed of running is the main way High-Runner mice achieve their increased running distance, especially for females (Girard *et al.*, 2001; Koteja and Garland, 2001). These data suggest that High-Runner mice have reduced function of dopamine, because the DAT blockers, which increase the function of dopamine, reversed the hyperactivity. However, the mechanism is still not known. For example, the High-Runner mice might produce less dopamine, have fewer dopamine receptors, or display reduced second messenger signaling in response to dopamine-receptor stimulation as compared with Control mice. These results prompted us to explore the dopamine pathway more closely in hopes of determining what specific alterations had taken place.

When dopamine is released, it interacts with recep-

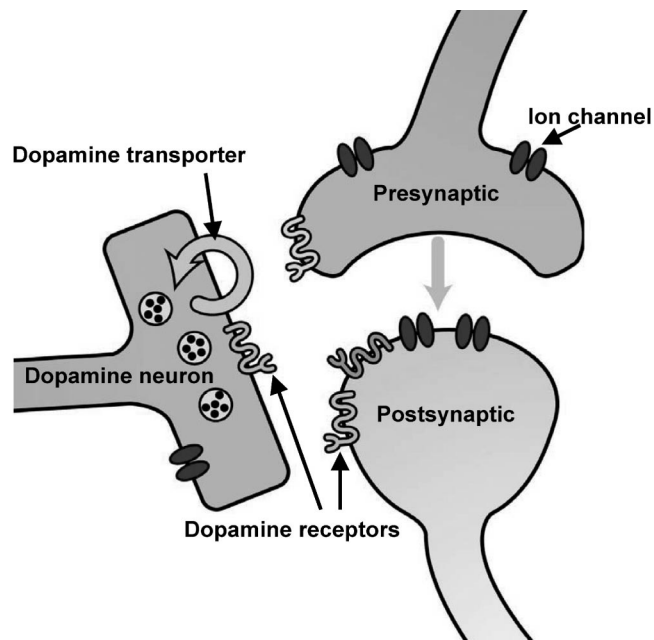


FIG. 2. Cartoon depicting how a dopamine neuron can modulate communication between two other neurons. The scenario might occur anywhere in the brain and the neurons being modulated by dopamine could be of many different types, but consider the following hypothetical example that takes place in the nucleus accumbens. A glutamate neuron (represented by “presynaptic”) projects into the nucleus accumbens from some other brain region (*e.g.*, prefrontal cortex). In response to firing of the presynaptic neuron, glutamate would be released and would bind to receptors on the “postsynaptic” neuron. The postsynaptic neuron might contain another neurotransmitter (*e.g.*, GABA). The GABA, postsynaptic neuron might become depolarized by the glutamate signal and send an action potential away from the nucleus accumbens to some other brain region (*e.g.*, the hippocampus). Dopamine would modulate this activity by interacting with receptors on both the pre- and postsynaptic neurons. Depending on the types of dopamine receptors that occur at these sites, dopamine could either increase or decrease the likelihood that the GABA neuron produces an action potential in response to the glutamate signal. Dopamine receptors affect properties of the ion channels through a complex second messenger system (Greengard *et al.*, 1999). The pattern of firing of the dopamine neuron, the dopamine transporter protein, and autoreceptors on the dopamine neuron itself would regulate the quantity of dopamine in the extracellular spaces.

tors on cells (see Fig. 2), causing signal transduction cascades that can affect properties of ion channels and expression of genes (Vallone *et al.*, 2000). Five different subtypes of dopamine receptors have been characterized, and these fall into two groups: D1-like (including the D1 and D5 receptors) and D2-like (D2, D3, D4 receptors). All 5 of these dopamine receptors are expressed in human and rodent brains (Jarvie and Caron, 1993). Therefore, the next goal was to determine whether High-Runner mice respond differently than Control mice to drugs that interact specifically with these receptor types.

The first dopamine-receptor drug that we tested was apomorphine, which interacts with all classes of dopamine receptors in a fashion similar to dopamine itself (Thal *et al.*, 1978). Apomorphine reduced wheel

running in both High-Runner and Control mice, but a higher dose was required in High-Runner mice (Rhodes and Garland, 2003). In other words, High-Runner mice were less sensitive than Controls to the behavioral effects of apomorphine. These data suggest that the function of dopamine receptors is reduced in High-Runner mice, and are consistent with the results of the DAT blockers. To identify whether the alteration was specific to D1- or D2-like receptors, we employed drugs that specifically prevent one or the other class of receptors from binding dopamine. Both SCH 23390, which specifically blocks only the D1-like receptors, and raclopride, which specifically blocks only the D2-like receptors reduced wheel running in both High-Runner and Control mice, but High-Runner mice were less sensitive than Controls to SCH 23390, whereas they were equally sensitive to raclopride (Rhodes and Garland, 2003). At first, these results might seem confusing because seemingly opposing treatments (apomorphine stimulates dopamine receptors and SCH 23390 and raclopride block dopamine receptors) both reduced wheel running in High-Runner and Control mice. However, if dopamine is necessary for wheel running, then it seems likely that it would have to function at a specific level and that a pharmacological manipulation that disturbs the system in any way would have the potential to interfere with the complex motor skills required for wheel running. Thus, we feel that the direction of the behavioral response is not as critical as the differential sensitivity between the lines for this interpretation. The fact that the High-Runners were less sensitive to the D1-like drug specifically implicates the D1 and D5 receptor subtypes in the High-Runner mice.

Reduced function of the D1-like dopamine signaling pathway could have many causes. The simplest hypothesis is that the structure (amino acid sequence) or expression (quantity or density) of the D1 or D5 dopamine receptors has changed in High-Runner mice. Different alleles for D1 and D5 receptors have been identified among inbred strains of mice (Mouse Genome Informatics database, The Jackson Laboratory, Bar Harbor, Maine) but whether such variation was present in the base population from which the High-Runner and Control lines were derived is not known. The hypothesis that allele frequencies for the D1 or D5 receptors have changed in High-Runner mice could be tested by sequencing the genes for these receptors in several High-Runner and Control mice to compare allele frequencies. This has not yet been done. Alternatively, differences in the expression of the D1 or D5 dopamine receptors could occur even though the structural genes are the same. This could be caused by a change in the DNA sequence of a promoter region or a change in "regulatory genes" such as those for transcription factors or proteins that affect the trafficking of the receptors from the cytoplasm to the cell membrane. Such regulatory genes could exist physically far away from the chromosomal location of the structural genes. An example of this phenomenon was observed

in Janowsky *et al.* (2001) where expression of DAT in the brains of different recombinant inbred strains was affected by the DNA sequence of regulatory genes located far away from the location of the DAT gene (Janowsky *et al.*, 2001). It was also recently demonstrated that selection for high alcohol withdrawal in mice resulted in an alteration in a gene for a protein (MPDZ) that affects the trafficking of receptors from the cytoplasm to the cell membrane (Shirley *et al.*, 2004). Other possible mechanisms of reduced D1-like dopamine signaling that do not involve changes in the structure or expression of dopamine receptors include differences in the numerous second messengers that are downstream of the receptors, structural differences in the anatomy of dopamine projections in the brain, or changes in other neurotransmitter systems that interact with dopamine.

In addition to dopamine, other neurotransmitter systems such as norepinephrine, glutamate, and GABA might have been altered by selective breeding for increased wheel running. GABA modulates dopamine concentrations and has been hypothesized to play a role in cocaine-induced locomotor stimulation in mice (Dewey *et al.*, 1997). The possible contribution of these other neurotransmitter systems in our wheel-running model awaits future study. A role for norepinephrine seems especially promising given that it is implicated in genetic hyperactivity in humans (Solanto *et al.*, 2001). Glutamate and GABA are also likely candidates because they play key roles in brain reward circuitry (Kelley and Berridge, 2002).

At present, we have some evidence *against* the hypothesis that serotonin or opiate systems have evolved in the High-Runner mice. Specifically, High-Runner and Control mice responded similarly to the serotonin transporter blocker, fluoxetine (Prozac) (Rhodes *et al.*, 2001), and to two different opioid receptor antagonists, naloxone and naltrexone (Li *et al.*, 2004). The lack of evidence for the evolution of the opioid system is particularly interesting given that it appears to play a key role in suppression of pain during exercise (Thoren *et al.*, 1990) and in brain reward (Van Ree *et al.*, 2000; Kelley and Berridge, 2002). Regarding brain reward, recently a hypothesis was put forward that dopamine regulates motivation for natural and drug rewards (*i.e.*, wanting), whereas opioids function in the perception of the reward itself (*i.e.*, liking) (Berridge and Robinson, 1998). It is possible that both High-Runner and Control mice perceive the same pleasure from wheel running (via an identical opioid system, as suggested by Li *et al.*, 2004), with the difference being that High-Runner mice are more motivated (via an altered dopamine system) than Control mice to seek the wheel-running reward.

Brain imaging

Results of the behavioral pharmacological studies suggested that dopamine function is altered in High-Runner mice. When dopamine is released into extracellular spaces it binds to receptors on both presyn-

aptic and postsynaptic neurons and thereby affects neuronal activity (Fig. 2). Dopamine cell bodies reside in the ventral midbrain but their axons extend to nearly all regions of the brain. Dense projections occur to the caudate-putamen and nucleus accumbens (Haglund *et al.*, 1979; Fallon, 1981), brain regions that play important roles in voluntary locomotion and motivation, respectively (Kelley and Berridge, 2002). Relatively more diffuse projections occur to the prefrontal cortex (Haglund *et al.*, 1979; Fallon, 1981), a brain region involved in motivation (Cardinal *et al.*, 2002). We hypothesized that the altered dopamine function might cause differential patterns of brain activation in High-Runner *versus* Control mice in these and other brain regions. For an initial test of this hypothesis, we measured activation of 25 brain regions (using immunohistochemical detection of c-Fos; see below) in High-Runner and Control mice (Rhodes *et al.*, 2003a).

Immunohistochemical detection of c-Fos (Fos-IR) is widely used to measure brain activation in rodents (Dragunow and Faull, 1989; Harris, 1998). It gives high resolution, down to single cells. Methods of brain imaging used in humans, such as Positron Emission Tomography (PET scanning) (*e.g.*, Childress *et al.*, 1999) or functional Magnetic Resonance Imaging (fMRI) (*e.g.*, Gottfried *et al.*, 2003), which track energy metabolism and/or patterns of blood flow in the brain, give much lower resolution. Fos-IR cannot be used in humans because subjects must be sacrificed. To conduct Fos-IR, brains must be removed, sectioned, and stained for the protein c-Fos. c-Fos is a transcription factor. Occasionally, when a neuron is stimulated a reaction cascade occurs that affects the expression of genes. One gene that is often immediately up-regulated, with a time course of reaching peak concentrations approximately 1 to 2 hours after stimulation is the protein c-Fos (Zangenehpour and Chaudhuri, 2002). c-Fos-containing protein complexes bind to promotor regions of target genes and change their expression. Not all neurons express c-Fos when they are stimulated, but many do (Herdegen and Leah, 1998; Ryabinin, 2000). A high amount of c-Fos staining in a neuron indicates that the neuron was activated within an approximately 90-min period leading up to sacrifice (for further explanation of how and why this works, see Dragunow and Faull, 1989). Activation in this case means that the neuron received either excitatory or inhibitory inputs that were associated with changes in second messenger activity (see Fig. 2). Neurons are scored positively for c-Fos if they display a level of c-Fos staining above some predetermined background threshold. c-Fos-positive nuclei are then counted within specific brain regions and the number is used as the index of neuronal activation.

We aimed to find brain regions putatively involved in the increased motivation for wheel running in the High-Runner mice (Rhodes *et al.*, 2003a). Realizing that differences in brain activation between High-Runner and Control mice might be related to locomotor activity *per se* rather than motivation, we designed an

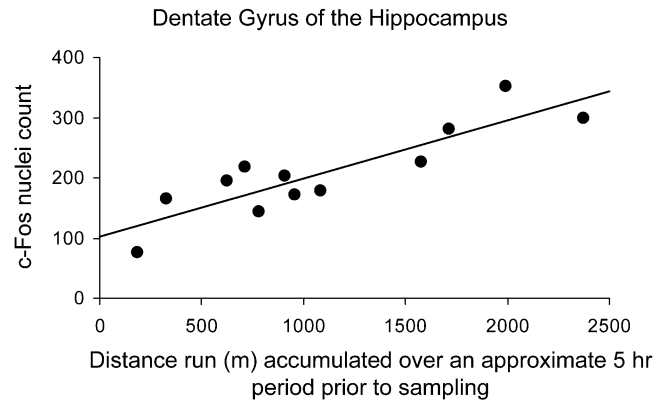


FIG. 3. Voluntary wheel running activates the dentate gyrus of the hippocampus, a region well known for its role in learning and memory. The effect is similar in High-Runner and Control mice. Relationship between the amount a mouse runs and number of c-Fos-positive nuclei in the dentate gyrus among mice from the Control lines. Modified from Figure 3 of Rhodes *et al.* (2003a).

experiment to separate these effects. Mice were given access to running wheels for 6 days, as in the normal selection protocol. On day 7, half the animals were prevented from accessing their wheels by placing a tile between the wheel-access tunnel and the cage (Blocked). Mice were sampled approximately 5 hours later, at a time when they are normally running at peak levels. From the perspective that wheel running is rewarding, and addictive, the blocked mice represent a group of animals in a state of withdrawal or “wanting” to run. The purpose of including the blocked treatment was to measure brain activation that might reflect differences in motivation for running without the confounding influence of acute effects of the wheel running itself. The other half of the mice were permitted continuous wheel access up to sampling (Runners). These mice were used to find brain regions involved in the expression of wheel running itself (Rhodes *et al.*, 2003a).

Of all 25 brain regions examined, the one that showed the strongest evidence of playing a role directly in the control of the locomotor activity itself was the dentate gyrus, which is a subregion of the hippocampus. In the dentate gyrus, the number of c-Fos positive nuclei was strongly related to how much a mouse ran prior to sampling. For example, an unselected Control mouse that ran 2,000 meters prior to sampling had an average of 3 times as many c-Fos positive nuclei as compared with one that ran 200 meters (see Fig. 3). Initially, this was surprising because the hippocampus is most well known for its role in learning and memory, not locomotion. However, our results are consistent with several intriguing studies that demonstrate hippocampal involvement in control of the intensity at which a behavior is performed (Morris and Hagan, 1983; Oddie and Bland, 1998; Slawinska and Kasicki, 1998). An interesting future study would be to lesion the dentate gyrus and see if it reduces voluntary wheel running (in particular, speed).

Dentate Gyrus of the Hippocampus

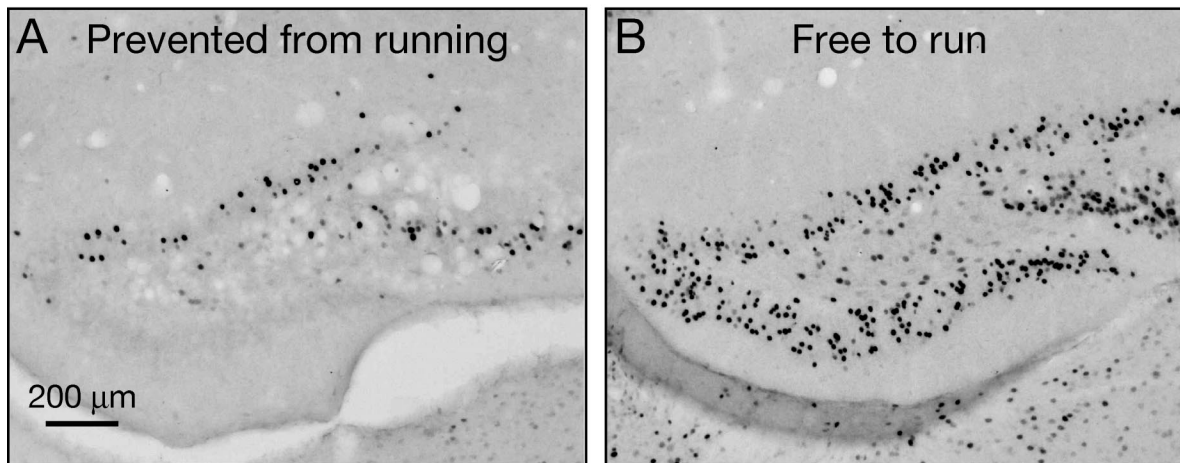


FIG. 4. The dentate gyrus of the hippocampus, a region well known for its role in learning and memory, is strongly activated by voluntary wheel running. Representative example of (A) a High-Runner mouse prevented from running and (B) a High-Runner mouse free to run. Neuronal activation is represented by number of c-Fos-positive nuclei, which appear as black dots in the photos. Modified from Figure 3 of Rhodes *et al.* (2003a).

The relationship between level of wheel running and activation of the dentate gyrus was steep for Control mice (Fig. 3), but appeared to reach a plateau in High-Runner mice (*i.e.*, a statistically significant interaction between line-type and distance run was observed, with the relationship being relatively more flat for High-Runner mice; see Fig. 3D in Rhodes *et al.*, 2003a). We interpreted this interaction not as evidence that the dentate gyrus is different in High-Runner and Control mice, but rather that there is a limit to the amount of activation that can occur in response to running which the High-Runner mice reach by virtue of their high running (Fig. 4). Further evidence that the dentate gyrus may not have evolved in the High-Runner mice is that it appeared to play little role in motivation for running. The dentate gyrus was relatively silent in mice that were blocked from running, and there were no differences between High-Runner and Control mice under this condition (Rhodes *et al.*, 2003a). Activation of the dentate gyrus may merely reflect the intensity of the locomotor activity after the animals have already decided how intensely they want to run.

We hypothesized that brain regions involved in reward and motivation would be among the most likely to have evolved in High-Runner mice (Rhodes *et al.*, 2003a). These include the caudate-putamen complex (which also plays a role in locomotion), nucleus accumbens, prefrontal cortex, and lateral hypothalamus. In all of these regions, we observed huge amounts of c-Fos in the blocked mice as compared to runners. Moreover, among those animals that were blocked from running, High-Runners displayed more c-Fos-positive nuclei than did Controls and/or the number of c-Fos nuclei was strongly correlated with the distance run on the *previous* day. Distance run on the previous day serves as an index of motivation in blocked mice, because it accurately predicts how much an animal

would want to run if it could (*i.e.*, wheel running is a highly repeatable behavior, see Fig. 2B in Rhodes *et al.*, 2003a). Activation of the sensory cortex followed a similar pattern as the nucleus accumbens, prefrontal cortex, and lateral hypothalamus even though the sensory cortex has not been previously associated with motivation or reward. Thus, future research is needed to investigate whether the sensory cortex indeed plays a role specifically in motivation for wheel running or whether it also plays a role in motivation for other rewards, such as those derived from food, sex or drugs of abuse. Interestingly, none of the putative motivation regions appeared to play a role in the locomotor activity itself because number of c-Fos nuclei in these regions was unrelated to how much a mouse ran prior to sampling in the separate group given continuous wheel access (Rhodes *et al.*, 2003a).

The lateral hypothalamus displayed an especially strong correlation between distance run the previous day and number of c-Fos-positive nuclei, which strongly implicates this region in controlling motivation for running (Rhodes *et al.*, 2003a). The lateral hypothalamus contains cells that secrete a protein neurotransmitter called “orexin” or “hypocretin.” Orexin neurons project widely throughout the brain and function in arousing the brain (España *et al.*, 2001). A promising future investigation would be to identify the phenotype of the cells that are activated in the lateral hypothalamus in association with motivation for voluntary wheel running to determine if those cells indeed contain orexin (*i.e.*, prevent the mice from running after 6 days continuous access and then stain the brain with antibodies to c-Fos and orexin to see if both proteins co-localize in the same neurons within the lateral hypothalamus). Orexin would represent an interesting candidate neuromodulator, in addition to dopamine, whose function might have evolved in High-Runner

TABLE 1. Mean (standard errors in parentheses) number of *c-Fos*-positive nuclei (indicator of brain activation) counted in the listed brain regions of Control and High-Runner mice, after an injection of saline or Ritalin (30 mg/kg) ($n = 2$ per group, 8 total).

Brain region	Saline		Ritalin	
	Control	High-runner	Control	High-runner
Caudate-putamen	33 (1.0)	50 (8.6)	625 (90.1)	880 (122.8)
Nucleus accumbens	38 (9.1)	53 (27.1)	233 (21.2)	308 (1.2)
Substantia nigra	166 (2.1)	196 (4.8)	403 (94.1)	380 (64.7)
Medial frontal cortex	362 (63.4)	266 (24.0)	405 (105.3)	805 (90.6)
Sensory cortex	562 (68.6)	333 (29.4)	440 (76.0)	760 (112.8)
Piriform cortex	292 (97.3)	250 (61.7)	340 (49.0)	368 (68.5)

Ritalin significantly increased brain activation in the caudate-putamen, nucleus accumbens, and substantia nigra in both Control and High-Runner mice ($P < 0.05$). In the medial frontal cortex and sensory cortex, Ritalin increased brain activation in High-Runner but not in Control mice, as indicated by a significant drug-by-line-type interaction ($P < 0.05$), and inspection of the means. No significant effect of Ritalin on brain activation of the piriform cortex occurred in either Control or High-Runner mice.

mice to produce increased motivation for running. On the other hand, the activity of orexin neurons could be controlled by dopamine neurons or other neurons influenced by dopamine's modulatory influence (see Fig. 2).

In addition to looking at patterns of brain activation in High-Runner and Control mice during running and when blocked from running, we also conducted a small study to examine patterns of brain activation in response to Ritalin. Recall that High-Runner and Control mice responded entirely differently to Ritalin and that the differential behavioral response appeared to be related to Ritalin's effect on the dopamine system (Rhodes and Garland, 2003). Thus, we hypothesized that the number of *c-Fos*-positive nuclei in specific brain regions that receive dopaminergic innervation (e.g., caudate-putamen, nucleus accumbens, medial frontal cortex) would differ between High-Runner and Control mice after an injection of Ritalin.

Female mice (age = 14 weeks, from generation 29 of the selection experiment) that were never exposed to running wheels were sacrificed after being injected with either 30 mg/kg Ritalin ($n = 2$ Control mice, $n = 2$ High-Runner mice) or saline ($n = 2$ Control mice, $n = 2$ High-Runner mice). The mice were housed in standard laboratory cages without wheels, and then sacrificed 2–3 hours after the injection, which was given 1 hour after lights off. The order of sacrifice alternated between High-Runner and Control mice; the first two mice received Ritalin, the next two saline, the next two Ritalin, and the last two saline. Immunohistochemical staining was performed as described in Rhodes *et al.* (2003a). We examined the caudate-putamen, nucleus accumbens, and medial frontal cortex because these regions play roles in locomotor activity and motivation and are innervated by dopamine neurons. We examined the substantia nigra because it contains the dopamine cell bodies whose axons project to the caudate-putamen. The sensory cortex and the piriform cortex were included as negative controls and were not expected to vary with the treatments. The exact locations in stereotaxic coordinates where the nuclei counting was conducted can be found in Rhodes *et al.* (2003a), as these same brain regions were examined for responses to wheel running. The data were

analyzed using a two-way ANOVA with factors line-type (High-Runner or Control), injection-type (30 mg/kg Ritalin or saline vehicle), and their interaction. No covariates were included in the model. SAS Proc Mixed was used but no random effects were specified as there was no replication of individuals within lines.

As expected, Ritalin increased the number of *c-Fos*-positive nuclei in the caudate-putamen (18-fold increase, $F_{1,4} = 86.85$, $P = 0.0007$), nucleus accumbens (6-fold increase, $F_{1,4} = 158.28$, $P = 0.0002$), and substantia nigra (2-fold increase, $F_{1,4} = 13.46$, $P = 0.02$) in both High-Runner and Control mice, as compared to the saline injection (Table 1). The Ritalin-induced activation of these brain regions likely reflects increased binding of dopamine to receptors on neurons in these regions because Ritalin increases extracellular concentrations of dopamine (Kuczenski and Segal, 1997; Huff and Davies, 2002).

Ritalin-induced brain activation differed in High-Runner *versus* Control mice in the medial frontal cortex (Fig. 5; Table 1) and sensory cortex as indicated by a significant interaction between treatment (Ritalin *versus* saline) and line-type (High-Runner *versus* Control) on number of *c-Fos*-positive nuclei (for the medial frontal cortex, $F_{1,4} = 10.30$, $P = 0.03$; for sensory cortex, $F_{1,4} = 12.50$, $P = 0.02$). In High-Runner mice, Ritalin increased the number of *c-Fos*-positive nuclei in the medial frontal cortex by approximately 200% and in the sensory cortex by 100%, whereas Ritalin had little effect in these regions in Control mice (12% increase in medial frontal cortex, 22% decrease in sensory cortex). In the piriform cortex, which showed similar levels of *c-Fos* staining as compared to the other regions, Ritalin had no significant effects ($F_{1,4} = 1.34$, $P = 0.31$) and High-Runner and Control mice did not differ ($F_{1,4} = 0.01$, $P = 0.92$). These results implicate the medial frontal cortex and sensory cortex in contributing to the differential behavioral effects of Ritalin in High-Runner *versus* Control mice (Fig. 1). Both regions receive dopamine innervation, but only the medial frontal cortex has been previously implicated in the behavioral effects of Ritalin (Solanto *et al.*, 2001). The possible involvement of the sensory cortex is a new finding. The fact that these two regions were also implicated in motivation for wheel running

Medial Frontal Cortex

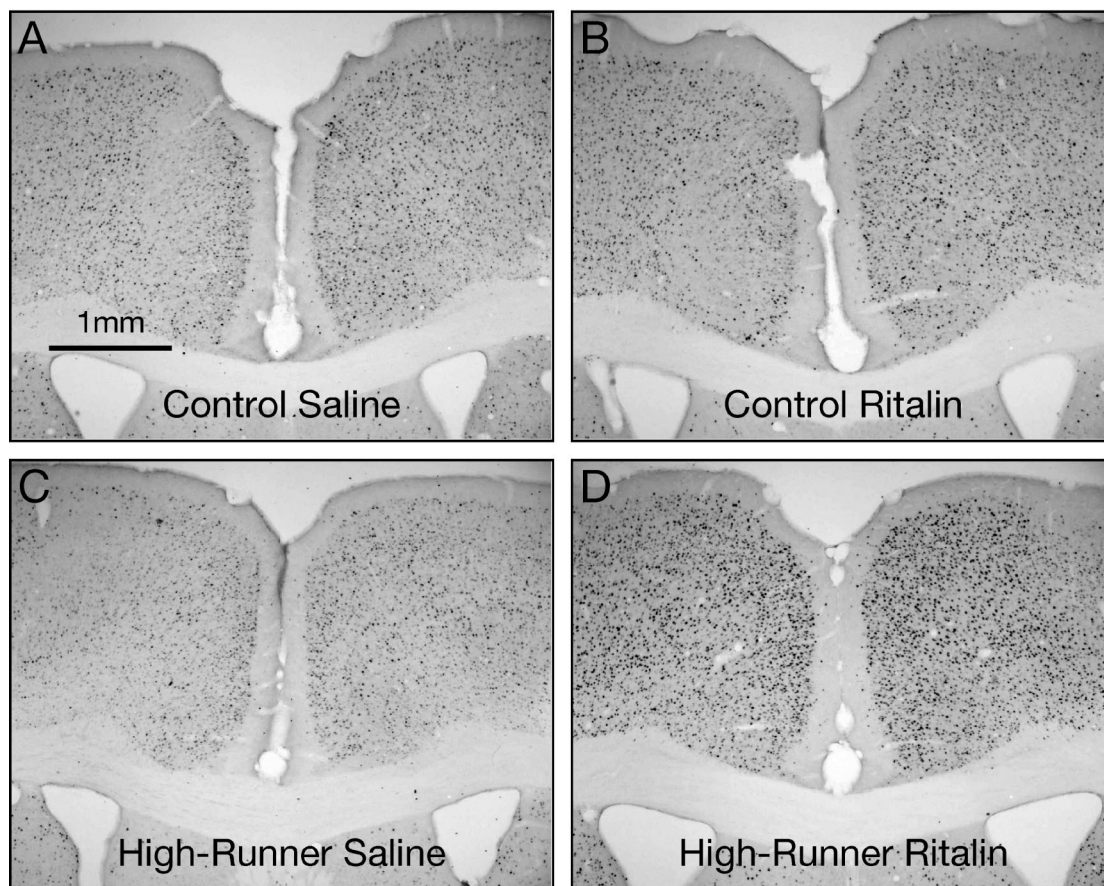


FIG. 5. Ritalin increased brain activation in High-Runner mice but not in Control mice in the medial frontal cortex, a region well known to play a role in motivation for natural rewards (Kelley and Berridge, 2002). Representative example of (A) a control mouse administered saline, (B) a control mouse administered 30 mg/kg Ritalin, (C) a High-Runner mouse administered saline, and (D) a High-Runner mouse administered 30 mg/kg Ritalin. Neuronal activation is indicated by number of c-Fos-positive nuclei, which appear as black dots. See Table 1 for quantification of results and text for statistics.

in a separate experiment when mice were blocked from accessing their wheels (Rhodes *et al.*, 2003a) is intriguing. Taken together, these data provide strong support for the hypothesis that the medial frontal cortex and sensory cortex are sites where alterations have taken place to increase motivation for voluntary wheel running in High-Runner mice.

Brain concentrations of dopamine

As discussed above, behavioral pharmacology suggested that High-Runner mice have reduced dopamine function (Rhodes *et al.*, 2001; Rhodes and Garland, 2003), and immunohistochemical detection of c-Fos identified brain regions that display differential neuronal activation in High-Runner *versus* Control mice associated with motivation to run (Rhodes *et al.*, 2003a). However, the mechanism by which dopamine function might be reduced to produce differential brain activation and increased motivation for running is not known. Reduced function of dopamine could be mediated by many different mechanisms, including re-

duced expression of dopamine receptors, changes in second messenger systems downstream of dopamine receptors, or reduced release of dopamine itself from dopamine neurons. In a preliminary investigation, we explored the possibility that High-Runner mice release less dopamine than Controls into the nucleus accumbens and caudate-putamen, two brain regions involved in motivation and brain reward, and that receive dense dopaminergic innervation.

Dopamine can be sampled in the extracellular spaces of the brain in live, behaving animals using microdialysis probes (Damsma *et al.*, 1992; Castner *et al.*, 1993; Hattori *et al.*, 1994; Paulson and Robinson, 1994; Salamone, 1996; Becker *et al.*, 2001) or carbon fiber electrodes implanted into the brain (Phillips *et al.*, 2003). However, these techniques are technically difficult and quite time consuming. Therefore, we chose a cruder method for the preliminary investigation. Specifically, we measured total concentrations of dopamine and its primary catabolite, DOPAC, in portions of the nucleus accumbens and caudate-putamen.

The ratio of DOPAC/dopamine has been used as an index of dopamine release, and it is correlated with dopamine release (as measured by microdialysis), at least under some conditions (Berridge *et al.*, 1999). We also measured serotonin and serotonin's primary catabolite, 5HIAA, to serve as negative controls, because these neurochemicals were not expected to differ between High-Runner and Control mice.

Concentrations of dopamine and DOPAC in the nucleus accumbens and caudate-putamen can vary with physical activity. For example, the speed of forced running on a straight treadmill is sometimes positively correlated with concentration of dopamine and DOPAC in the nucleus accumbens and caudate-putamen of rats (Freed and Yamamoto, 1985) but see (Hattori *et al.*, 1994). Therefore, we designed an experiment that would allow us to control running speed to determine whether High-Runner mice release less dopamine per unit speed of running. Thirty-four female mice (from generation 27 of the selection experiment) were randomly assigned to 4 treatments: sedentary ($n = 8$, 3 individuals from Control lines, and 5 from High-Runner lines), 0.5 km/hr ($n = 9$, 4 from Control lines and 5 from High-Runner lines), 1 km/hr ($n = 8$, 4 from Control lines and 4 from High-Runner lines), and 1.5 km/hr ($n = 9$, 4 from Control lines and 5 from High-Runner lines). All mice, except those in the sedentary treatment, were forced to run on a treadmill for 20 minutes at the assigned speeds on 4 consecutive days. Sedentary mice were left in their home cages, undisturbed. The first 3 days were considered training sessions to familiarize the mice with the treadmill protocol and to eliminate novelty responses. Treadmill sessions occurred during the day (1430–1800 hr; photoperiod was 700–1900). In the actual trial on the fourth day, after 20 min of treadmill running, mice were decapitated and their brains quickly removed and placed on ice. Animals in the sedentary treatment were simply removed from their cages and decapitated. A portion of the nucleus accumbens and caudate-putamen were dissected, weighed, and placed into a microcentrifuge tube on dry ice. Samples were stored at -80 before they were assayed for dopamine, DOPAC, serotonin, and 5HIAA by high performance liquid chromatography with electrochemical detection (HPLC/ec) following Berridge *et al.* (1999). SAS Proc Mixed was used to analyze the data in a linear model that included factors, line-type (High-Runner or Control), treadmill running speed (0, 0.5, 1, or 1.5 km/hr), and their interaction. Data were also analyzed with treadmill running speed entered as a continuous variable to examine correlations between running speed and neurochemical concentration. Replicate line (nested within line-type) was always entered as a random effect. In addition, because the animals were run over a period of 4 weeks, and neurochemicals such as dopamine can begin to degrade in the -80 freezer over that period, the week animals were processed (batch) was entered as a blocking factor in the model (see Rhodes, 2002). Finally, the time it took to dissect a

brain region and place it onto dry ice (tissue time) was entered as a covariate, as neurochemicals could begin to degrade during that time, as well.

None of the neurochemicals, nor the ratios (*e.g.*, DOPAC/dopamine), differed significantly (all $P > 0.05$) between High-Runner and Control mice for any treatment (sedentary or 0.5, 1, 1.5 km/hr) in the caudate-putamen or nucleus accumbens. Moreover, we found no correlation between concentration of any of the neurochemicals and treadmill running speed. It is possible that differences might have been found had we looked at other brain regions, such as the prefrontal cortex or the lateral hypothalamus (see section on Brain Imaging). On the other hand, High-Runner and Control mice might release dopamine at the same rate and what causes the differential dopamine function is the way neurons respond to the dopamine after it has been released (*e.g.*, altered expression or molecular structure of receptors or dopamine reuptake protein, or changes in the second messenger systems that respond to the dopamine signals; see Fig. 2).

Exploration of the stress axis

When mice are stressed, neurons within a region of the brain called the hypothalamus release a protein neuromodulator called "corticotropin-releasing hormone" (CRH) into the brain and into the blood (Smailin *et al.*, 2001). In the blood, CRH acts on the pituitary gland to stimulate release of adrenocorticotropic hormone (ACTH), which subsequently causes the adrenal glands to release corticosterone (CORT) into the blood. CORT has many functions to help the body deal with stress. Plasma concentrations of CORT have been used routinely to measure levels of stress in rodents (*e.g.*, references in Girard and Garland, 2002). It turns out that High-Runner mice have higher circulating CORT levels than Control mice, whether housed with or without wheels (Girard and Garland, 2002). Altered basal CORT levels might result from the altered dopamine function because dopamine neurons can modulate CRH neurons (Eaton *et al.*, 1996). On the other hand, the altered basal CORT level could play a causal role in the increased voluntary wheel running. CORT and CRH can affect neuronal activation throughout the brain (Nestler *et al.*, 1989; Clark *et al.*, 1991; Da Costa *et al.*, 1997; Dube *et al.*, 2000; Stamp and Herbert, 2001). It is possible, therefore, that increased release of CRH and/or CORT affects the function of dopamine neurons, and/or alters neuronal activation of brain regions involved in motivation and reward. This hypothesis has not been explored beyond demonstrating that the region of the hypothalamus that contains CRH neuron cell bodies (the paraventricular hypothalamic nucleus) is more active in High-Runner than Control mice both when mice are running and when they are prevented from running (Rhodes *et al.*, 2003a). Future investigations will be required to test the hypothesis that increased release of CRH is necessary for the increased voluntary wheel running. One possible study would be to administer a drug that blocks CRH action

(Goeders and Guerin, 2000) to determine whether it reduces the high wheel running exhibited by the High-Runner mice. Similar studies could explore whether the elevated CORT levels are necessary for the elevated wheel running.

Neurogenesis and learning

Voluntary wheel running increases the expression of brain derived neurotrophic factor (BDNF) in the hippocampus (Neeper *et al.*, 1995). When this report first came out it was very exciting because the hippocampus is most well known for its role in learning and memory and BDNF functions to protect and strengthen connections in the brain (Oliff *et al.*, 1998; Johnson *et al.*, 2003). Moreover, in adult mammals, the hippocampus is unusual in that it is able to regenerate its own neurons (Gage *et al.*, 1998; Gage, 2000), and voluntary wheel running also increases the number of new neurons that form in the hippocampus of adult mice (van Praag *et al.*, 1999a; Rhodes *et al.*, 2003b). Voluntary wheel running can also enhance learning (van Praag *et al.*, 1999b; Anderson *et al.*, 2000), and it has been theorized that the learning enhancement may come from the BDNF and/or growth of new neurons (van Praag *et al.*, 1999b; Rhodes *et al.*, 2003b). Because High-Runner mice exercise more than Control mice, we hypothesized that they would display more BDNF and neurogenesis and, therefore, learn how to navigate a maze faster than Control mice. We found that High-Runner mice do indeed display more exercise-induced BDNF and neurogenesis than Control mice, but they display impaired learning as compared with Control mice when both are housed with wheels (Rhodes *et al.*, 2003b). It is possible that their apparent high motivation to exercise interferes with their attention to complex cues required for learning. The impaired learning in the High-Runner mice despite increased BDNF and neurogenesis also casts doubt on the theory that the functional significance of exercise-induced BDNF and neurogenesis is to enhance learning (van Praag *et al.*, 1999b).

Recall that wheel running activates the hippocampus (Rhodes *et al.*, 2003a). We hypothesize that the functional significance of exercise-induced BDNF and neurogenesis may be to increase the capacity of the hippocampus to become activated during exercise. It is also possible that the intense activation of the hippocampus during exercise actually stresses the hippocampus to the point where some neurons become damaged or die (Ramsden *et al.*, 2003; Schauwecker, 2003). If this were true, it might explain why the hippocampus is one of the few regions of the brain that has evolved the capacity to regenerate its own neurons. No one has ever tested whether high levels of wheel running actually kill neurons in the hippocampus. Therefore, an exciting future study would be to determine whether neuronal death in the hippocampus is correlated with level of wheel running. If neuronal death is correlated with level of exercise, then that would provide strong support for the theory that the

functional significance of exercise-induced BDNF and/or neurogenesis is in restoration of the hippocampus, rather than in learning enhancement.

BIOMEDICAL IMPLICATIONS

Exercise addiction and mental health

The neurobiological profile of High-Runner mice suggests that they are dependent on exercise, akin to an exercise addiction (Aidman and Woollard, 2003). When High-Runner mice are prevented from conducting their daily exercise routine on running wheels, a similar pattern of brain activation occurs as when rats are prevented from getting their daily fix of cocaine, nicotine or morphine (Neisewander *et al.*, 2000; Schroeder *et al.*, 2000, 2001; Rhodes *et al.*, 2003a). Others have suggested that wheel running produces a natural reward (Belke, 1996; Sherwin, 1998; Nestler *et al.*, 2001; Werme *et al.*, 2002; Eikelboom and Lattanzio, 2003). Thus, the High-Runner mice appear to have developed an increased craving, motivation and/or dependence for this exercise reward in a similar way that a rat can be made dependent on a drug of abuse (Rhodes *et al.*, 2003a). The same neurotransmitter—dopamine—is implicated in both the process of craving or “wanting” drugs (Berridge and Robinson, 1998; Joseph *et al.*, 2003) and motivation for wheel running in High-Runner mice (Rhodes *et al.*, 2001; Rhodes and Garland, 2003). Moreover, GBR 12909, which is currently in clinical trials for use as a substitution therapy for cocaine abuse (<http://www.clinicaltrials.gov/ct/show/NCT00051896?order=10>), also appears to substitute for wheel running because it reduces the speed but not duration of wheel running in High-Runner mice (Rhodes *et al.*, 2001).

The implication for human health is that physical activity can be addictive in the sense that it can induce withdrawal if exercise is denied (see Aidman and Woollard, 2003, for a human example). Moreover, the exercise addiction in our mice is genetically based, which suggests the extrapolation that some individual human beings may carry genes that make them especially prone to this form of addiction. One might think that addiction to physical activity would have positive biomedical implications because physical activity is generally good for the body, the brain, and a sense of well-being (Scully *et al.*, 1998; Cotman and Berchtold, 2002). However, what the neurobiological profile of the High-Runner mice tells us is that extreme levels of exercise may have deleterious effects. For example, the High-Runner mice have chronically elevated corticosterone levels, whether they are exercising or not (Girard and Garland, 2002), which can damage the body and brain (Sapolsky, 1996, 2000). Although a normal amount of exercise can improve learning (van Praag *et al.*, 1999b; Anderson *et al.*, 2000; Rhodes *et al.*, 2003b), the high levels of exercise in High-Runner mice is associated with impaired learning when they have access to running wheels (Rhodes *et al.*, 2003b). The impairment may come from an overactive hip-

pocampus. Wheel-running exercise strongly activates the hippocampus (*e.g.*, see Fig. 3), and although the hippocampus may be normally able to respond by growing new neurons and producing chemicals that strengthen pre-existing connections, the capacity for regeneration and strengthening may be limited or overwhelmed by the high levels of exercise in High-Runner mice (Rhodes *et al.*, 2003b). Thus, results from our selection experiment suggest that exercise should be conducted in moderation.

The idea that exercise produces a natural reward that resembles the reward associated with drugs of abuse is intriguing and may provide some key insights into the pathology of drug addiction (Kelley and Berridge, 2002). The human brain evolved to respond to natural rewards, such as food, sex, and exercise, long before synthetic drugs of abuse were available. The perception of pleasure from eating, engaging in sexual activity or exercising represents part of an evolutionary "strategy" to ensure that these behaviors are expressed. One possibility is that drugs mimic natural rewards, thus causing drug-seeking behavior to be reinforced even though drugs generally do not provide a benefit in terms of Darwinian fitness (Wise, 2002). Alternatively, drugs might alter natural brain reward circuitry resulting in increased motivation to obtain drugs in a fashion that is disproportionate to the pleasurable effect of the drugs (Nesse and Berridge, 1997; Berridge and Robinson, 1998). This might explain why users often seek drugs even though they produce aversive side effects, such as paranoia or nausea. By including responses to such natural rewards as exercise in addiction research, it may be possible to identify what makes drug craving different from natural craving. That would be an important first step toward development of a pharmaceutical therapy designed to target the pathology.

Attention deficit hyperactivity disorder

High-Runner mice share many features in common with human ADHD, which suggests that they may represent a useful model to study the neural and genetic basis of certain features of ADHD (Rhodes *et al.*, 2001; Rhodes and Garland, 2003; Rhodes *et al.*, 2003a), or vice versa! Similar to human subjects with ADHD, High-Runner mice are hyperactive (Rhodes *et al.*, 2001) and their hyperactivity is largely genetically determined. We consider the High-Runner mice to be hyperactive because they run almost three times as far as Control mice in running wheels and are more active in photobeam cages (Rhodes *et al.*, 2001; Garland, 2003). As compared with 13 species of wild murid rodents, the High-Runner mice are among the highest runners, but not *the* highest (see Fig. 4 in Garland, 2003; also see Swallow *et al.*, 2005). This is important because it demonstrates that hyperactivity is a relative concept (*i.e.*, the designation depends on what you use as the reference population). The hyperactivity in both High-Runner mice (Garland, 2003) and ADHD (Todd, 2000) is likely caused by many genes interacting in

complex ways with each other and with the environment. ADHD is most apparent in the habituated environment (*e.g.*, at home or at school). At the doctor's office it is difficult to diagnose ADHD (Sleator and Ullmann, 1981). Thus, medical doctors rely heavily on reports from parents and teachers. Consistent with this profile, High-Runner mice are hyperactive in their home-cage (Rhodes *et al.*, 2001) but not in novel, "stressful" environments (Bronikowski *et al.*, 2001). In addition, ADHD children (Solanto *et al.*, 2001) and High-Runner mice display motor impulsiveness (Girard *et al.*, 2001) (short bursts of physical activity with short interbout intervals). It has been hypothesized that reduced function of dopamine and altered neuronal activity in the prefrontal cortex underlie ADHD (Solanto *et al.*, 2001), and both of these features appear to occur in High-Runner mice (Rhodes *et al.*, 2001; Rhodes and Garland, 2003; Rhodes *et al.*, 2003a). Finally, Ritalin is one of the most widely used pharmaceuticals to treat ADHD (Solanto *et al.*, 2001), and it ameliorates the hyperactivity in High-Runner mice (Rhodes and Garland, 2003). In particular, Ritalin makes wheel-running of High-Runner mice more similar to Controls by reducing the speed, not duration, of wheel running (Rhodes and Garland, 2003). Moreover, Ritalin activates the medial frontal cortex differently in High-Runner *versus* Control mice (Table 1, Fig. 5). Future investigation of the mechanism for the differential effect of Ritalin on frontal cortical activation in High-Runner *versus* Control mice could shed light on mechanisms of action of Ritalin in correcting hyperactivity.

One key feature of ADHD is inattention (Solanto *et al.*, 2001), and we do not yet know if the High-Runner mice display impaired attention. Thus, for future development of the High-Runner mice as a model of ADHD, it will be important to test them on a task designed to measure attention, such as the go/no-go task (Eagle and Robbins, 2003). High-Runner mice housed with wheels displayed impaired learning in the Morris water maze (Rhodes *et al.*, 2003b), so it is likely they will display impairments in other tasks that require attention and learning. Although the Morris water maze was not designed to measure attention, successful performance does require attention to visual cues and, therefore, it is possible that the learning deficit was caused by an attention impairment in High-Runner mice. A promising future direction in the development of High-Runner mice as a model of ADHD, would be to determine whether Ritalin rescues the learning deficit observed in the Morris water maze or other tests that involve attention and learning.

Taken at face value as a model of ADHD, the High-Runner mice suggest that the genetic hyperactivity in ADHD is caused by reduced function of D1-like receptors (Rhodes and Garland, 2003) and altered physiology of the lateral hypothalamus, caudate-putamen, and medial frontal cortex (Rhodes *et al.*, 2003a). Brain imaging studies in humans support a role for the caudate-putamen and frontal cortex in ADHD (as reviewed in Castellanos, 2001), but to the best of our

knowledge, the lateral hypothalamus has never been implicated in ADHD. Thus, a putative role for the lateral hypothalamus in ADHD is a new insight from our model that deserves further investigation.

Like all models, an animal model of a human mental disorder never represents all features of the disorder, and as such it is noteworthy to mention inconsistencies between human ADHD and the High-Runner mice. Human linkage studies have identified an association between the incidence of ADHD and allelic variation in the gene for the D4 receptor (for a meta analysis, see Faraone *et al.*, 2001). The D4 gene is highly polymorphic (*i.e.*, many alleles exist) in humans (Faraone *et al.*, 2001), but only a few polymorphisms have been found in mice (Scott *et al.*, 1995). Therefore, it is unlikely that allelic variation in the D4 receptor is associated with hyperactivity in our model. However, a recent microarray study identified a trend for increased expression of the D4 receptor in the hippocampus of High-Runner relative to Control mice (Bronikowski *et al.*, 2004). Therefore, it is possible that the D4 receptor plays a role in the hyperactivity of High-Runner mice via a genetic alteration in a promoter region or transcription factor that ultimately increases D4 expression. However, involvement of possibly increased expression of D4 receptors in the phenotypic difference between High-Runner and Control mice is not supported by pharmacology because High-Runner mice responded similarly to at least some agents that blocked D4 receptors (Rhodes and Garland, 2003).

Another noteworthy difference between High-Runner mice and ADHD is that ADHD occurs at a higher incidence in males than in females (Solanto *et al.*, 2001), whereas the fold increase in wheel running, relative to Control lines, is virtually identical in High-Runner males and females (Koteja and Garland, 2001; Garland, 2003). Female house mice in general run more than males, and this phenomenon occurs in both our High-Runner and Control lines (Koteja and Garland, 2001; Garland, 2003). It is not yet known what underlies the sex difference in either ADHD (Biederman *et al.*, 1994, 2002) or wheel running. It may turn out that the sex difference in ADHD is not a biological phenomenon but rather a sociological one: a tendency for over-diagnosis in males relative to females because males are given more leeway to act out than females.

A unified theory of addiction

We have argued that High-Runner mice display many features in common with two distinct mental disorders: addiction and ADHD. We propose that their may be an intriguing connection here. If physical activity can be naturally rewarding and addictive, then perhaps ADHD can be considered a kind of an addiction, an addiction to the reward elicited by physical activity itself. By definition, addicts compulsively seek their reward of choice, devaluing other stimuli unrelated to acquiring the reward. Hence, ADHD subjects may have trouble concentrating on problems that require them to sit still for prolonged periods of time

because they have uncontrollable urges to move, which distracts their attention. A unified theory of addiction may be a useful construct in future explorations of the neurobiological basis of ADHD. One intriguing piece of clinical evidence that is consistent with our theory that ADHD is a form of an addiction is that ADHD children have a much higher incidence of becoming addicted to drugs than normal children (Comings, 1994; Solanto *et al.*, 2001). Their brains may simply be wired in a way that makes them susceptible to addictions of all kinds. Evidence from our work and many others on ADHD suggest that this wiring likely involves a specific alteration of the dopaminergic system, and hyperexcitability of brain regions involved in incentive motivation for reward. Future studies with the High-Runner mice hold promise for identifying the specific genes and biochemical pathways that contribute to risk for developing an addiction to physical activity.

CONCLUDING REMARKS

Selective breeding represents a powerful tool for integrative neuroscience research. With selective breeding, the investigator can shape behavior to reflect specific features or symptoms of a mental disorder. In our case, mice were bred to display high levels of voluntary wheel running. These mice were then used to identify neurobiological features that distinguish High-Runner mice from their Controls. The research has enabled insights into the underlying neural basis of genetic hyperactivity, addiction to natural rewards, and the relationship between exercise and learning. The future holds great promise for this model. With future research we may be able to discover how the dopamine system has evolved to produce hyperactivity, what specific combinations of genes predispose mice to become hyperactive, and why hyperactive mice allowed to exercise at high levels have difficulty learning despite increased generation of new neurons in the brain.

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