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Patterns of Brain Activity Associated With Variation in Voluntary Wheel-Running Behavior

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Rodents spontaneously run on wheels, but what underlies variation within and between species is unknown. This study used Fos immunoreactivity to compare brain activity in mice selectively bred for high wheel running (S) versus control (C) mice. Mice ran for 6 days, but on Day 7, half the mice were prevented from running. A strong positive correlation was found between running distance and Fos in the dentate gyrus of C runners that was lost in S runners. In mice prevented from running, Fos was higher in S than in C in the lateral hypothalamus, medial frontal cortex, and striatum. Results implicate specific brain regions in motivation to run and others in control of the intensity of the locomotor behavior itself.

Voluntary wheel running is one of the most widely studied behaviors in laboratory rodents, yet the underlying cause of variation in this behavior is not known (Sherwin, 1998). Recently, it has been proposed that wheel running is naturally rewarding and addictive (Belke, 1996; Belke & Belliveau, 2001; Iversen, 1993; Lett, Grant, Byrne, & Koh, 2000; Nestler, Barrot, & Self, 2001; Werme et al., 2002). Thus, differences in the appetitive value of wheel running may underlie variation in the behavior. On the other hand, differences in exercise capacity or perception of the aversive effects of exercise (e.g., pain) might determine levels of voluntary wheel running (Garland, 2003; Sherwin, 1998).

A recent study used selective breeding to increase voluntary wheel-running behavior in four replicate lines of house mice (S lines), while also maintaining four unselected (randomly bred) lines to serve as controls (C lines; Swallow, Carter, & Garland, 1998). The original goal was to provide a novel model with which to study the role of exercise physiology in voluntary running (Garland, 2003). However, surprisingly few exercise-related genetic adaptations have been found (Dumke et al., 2001; Garland et al., 2002; Girard & Garland, 2002; Houle-Leroy, Garland, Swallow, & Guderley, 2000; Houle-Leroy, Guderley, Swallow, & Garland, 2003), suggesting that the alteration in behavior has primarily resulted from changes at the level of the central nervous system (Rhodes et al., 2001; Rhodes & Garland, 2003). The behavioral profile of S mice is characterized by motor impulsivity (short bursts of activity with short interbout pauses; Girard, McAleer, Rhodes, & Garland, 2001), hyperactivity in photobeam cages (Rhodes et al., 2001), and differential sensitivity to dopamine drugs (Rhodes et al., 2001; Rhodes & Garland, 2003).

The aim of this study was to identify brain regions that play a role in the differential wheel running of S mice as compared with C mice. To achieve this objective, brain activity was compared between S and C mice by detection of the immediate early gene product, Fos (Harris, 1998; Sagar, Sharp, & Curran, 1988). Fos is transiently expressed in response to neuronal stimulation, and thus reflects short-term changes in brain activity (Harris, 1998; Sagar et al., 1988). Because we hypothesized that differences between the S and C mice would be greatest at the time of normal selection of breeders, all mice were given 6 days of wheel access in accordance with the standard selection protocol (see Swallow et al., 1998). On the test day (Day 7), half the mice were prevented from running by the placement of a tile between the wheel-access tunnel and the cage. Sampling occurred approximately 5 hr later, at a time when mice are normally running at peak levels. The other half were permitted continuous wheel access until the time of sampling. From the perspective that wheel running is rewarding and addictive (Belke, 1996; Belke & Belliveau, 2001; Iversen, 1993; Lett et al., 2000; Nestler et al., 2001; Werme et al., 2002), 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 activity that might reflect differences in the appetitive value of wheel running in S and C mice without the confounding influence of acute effects of the wheel running itself.

Previous studies have used Fos in conjunction with forced treadmill running in rats to identify brain regions involved in exercise (Iwamoto, Wappel, Fox, Buetow, & Waldrop, 1996; Jordan, 1998; Liste, Guerra, Caruncho, & Labandeira-Garcia,

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1997; Oladehin & Waters, 2001). The brain regions identified by these studies, including the lateral hypothalamus (LH), cuneiform nucleus (CnF), pedunculopontine tegmental nucleus (PPTg), pontine nucleus (Pn), lateral periaqueductal gray (LPAG), caudate-putamen (CPu), dentate gyrus (DG), and hippocampal subregions (CA2 and CA3), were hypothesized to differ in S versus C mice with continued wheel access (runners) because S runners exercise more than C runners. Separate studies have used Fos to identify brain regions that are activated when drugs of abuse or natural food rewards are denied, with the aim of identifying brain regions involved in craving or relapse (Schroeder, Binzak, & Kelley, 2001; Schroeder, Schiltz, & Kelley, 2003). The brain regions identified by these studies, including prefrontal cortex (PFC), medial frontal cortex (MFC), and nucleus accumbens (NAc), were expected to differ in S versus C mice that were blocked from exercising because S mice were hypothesized to be more dependent on wheel running or to perceive wheel running as having a greater appetitive value than C mice.

To the best of our knowledge, this is the first study to use Fos to examine short-term changes in brain activity associated with variation in voluntary wheel running in mice. Other studies have investigated the role of the transcription factor, ΔFosB, in regulating wheel running in mice and rats (Nestler et al., 2001; Werme et al., 2002). However, ΔFosB is much longer-lasting than Fos (Nestler et al., 2001) and therefore was not used in this study to investigate short-term changes in brain activity associated with variation in wheel running. All other reports of Fos and wheel running aimed to characterize sleep–wake cycles associated with circadian rhythms and focused on the suprachiasmatic nucleus (SCN) rather than regions involved in locomotor behavior and motivation (Amy, Chari, & Bult, 2000; Mikkelsen, Vrang, & Mrosovsky, 1998; Smale, McElhinny, Nixon, Gubik, & Rose, 2001). The specific aims of this study were to (a) compare patterns of Fos-immunoreactivity (Fos-IR) between S versus C mice during wheel running and (b) compare patterns of Fos-IR between S versus C mice denied wheel running in order to find putative sites of alterations associated with high motivation for wheel running.

Method

Subjects

We studied mice from Generation 29 of an artificial selection experiment for high voluntary wheel-running behavior. As described previously (Swallow et al., 1998), the original progenitors were outbred, genetically variable laboratory house mice (*Mus domesticus*) of the Hsd:ICR strain (Harlan –Sprague–Dawley). After two generations of random mating, mice were randomly paired and assigned to eight closed lines (10 pairs in each). In each subsequent generation, when the offspring of these pairs were 6–8 weeks old, they were housed individually with access to a running wheel for 6 days. Daily wheel-running activity was monitored by an automated system. In the four S lines, the highest running male and female from each family were selected as breeders to propagate the lines to the next generation. Wheel running was quantified as the total number of revolutions run on Days 5 and 6 of the 6-day test. In the four C lines, a male and a female were randomly chosen from each family. Within all lines, the chosen breeders were randomly paired, except that sibling matings were not allowed.

The National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80–23, revised 1996) was followed, and all experiments were approved by the University of Wisconsin Animal Care Committee. All efforts were made to minimize the number of mice used and their suffering. Throughout the selection experiment and during this study, water and food (Laboratory Rodent Diet 8604, Harland Teklad, Madison, WI; after Generation 23, breeding females were given Harlan Teklad Mouse Breeder Diet 7004) were available ad libitum. Rooms were controlled for temperature (~22 °C) and photoperiod (12-hr light–dark). During the regular selection experiment, lights were turned on at 0700 and off at 1900. However, the mice used in the current experiment were switched to a schedule in which lights were turned on at 0400 and off at 1600. The schedule was switched at the time when parents were paired. Thus, the mice used in this study were born and continuously maintained on the 0400–1600 schedule.

Experimental Treatments

To supply mice for the experiments presented here, Generation 28 parents were allowed to produce a second litter (i.e., Generation 29). We studied males to avoid possible effects of estrous cycle. All lines were equally represented ($N = 48$; 6 mice per line). The mice were housed individually with access to large, rat-sized running wheels (1.12-m circumference) for 6 days, starting at approximately 40 days of age, following the standard selective breeding protocol (Swallow et al., 1998). On Day 7, 2 hr before lights off, half of the mice ($n = 24$) were blocked from reaching their wheel (balanced within lines) by means of a tile that was quietly wedged between the cage and the wheel-access tunnel. We decided to use this method rather than lock the wheels (i.e., prevent rotation) and allow the mice to enter the locked wheels because a previous study demonstrated that the mice climb in their wheels when they are locked, and we wished to prevent the mice from exercising as much as possible (Rhodes, Koteja, Swallow, Carter, & Garland, 2000). Mice were perfused between 1.5 and 2.5 hr after lights off on Day 7, so that Fos-IR would indirectly reflect neuronal activity (Ji & Rupp, 1997) during a period when mice normally exhibit peak activity on running wheels (Girard et al., 2001; Girard & Garland, 2002). In order to fix brains within the 1-hr window, we processed 4 mice each day. Consequently, mice were placed on running wheels in a staggered fashion, 4 per day, over 12 days. The 4 mice in a batch always included one C runner, one C nonrunner, one S runner, and one S nonrunner, and the order in which the 4 mice were sampled was randomized each day.

Brain Regions

The brain regions listed in Table 1 were chosen prior to data collection. Most were chosen because they have been implicated in motivation, addiction, or locomotor behavior on the basis of the literature. The SCN (see Hochstetler, Garland, Swallow, Carter, & Bult-Ito, in press), paraventricular hypothalamic nucleus (PVN), piriform cortex (Pir), and visual cortex (V1) were included as negative controls that were not expected to vary with the treatments.

Immunohistochemistry

Mice were deeply anesthetized with sodium pentobarbital and then perfused transcardially with 4% (wt/vol) paraformaldehyde (100 ml each). Brains were removed, postfixed in 4% paraformaldehyde overnight at 4 °C, and placed in 30% (wt/vol) sucrose for 2 days. A sliding microtome (Leica) was used to cut coronal sections (40 μm), which were placed into tissue cryoprotectant solution (phosphate-buffered solution containing 30% sucrose, 30% [wt/vol] ethylene glycol, and 10% [wt/vol] polyvinylpryrrolidone), then stored at −20 °C. Immunohistochemistry for Fos was performed on free-floating sections in a phosphate buffering solution containing 0.2% Triton X-100 (following Gammie & Nelson, 2001). Tissue was incubated for 2 days at 4 °C with rabbit anti-Fos (Oncogene Research Products, Cambridge, MA), 1:20,000. To visualize the antibody,

Table 1
Brain Regions Chosen Prior to Data Collection

Abbreviation	Brain region
DG	Dentate gyrus of hippocampus
CA2/3	CA2/CA3 of hippocampus
BNST	Bed nucleus of the stria terminalis
MEnt	Entorhinal cortex
CPu	Caudate putamen
PFC	Prefrontal cortex
MFC	Medial frontal cortex
NAc	Nucleus accumbens
Pir[a]	Piriform cortex
AMY	Amygdala
PVA	Paraventricular thalamic nucleus
VA	Ventral anterior thalamic nucleus
S1[a]	Sensory cortex, trunk region
LH	Lateral hypothalamus
SN	Substantia nigra
LPAG	Lateral periaqueductal gray
DR	Dorsal raphe nucleus
CnF	Cuneiform nucleus
PPTg	Pedunculopontine tegmental nucleus
Pn	Pontine nucleus
PVN[a]	Paraventricular hypothalamic nucleus
DH	Dorsal hypothalamic nucleus
SCN[a]	Suprachiasmatic nucleus
SC	Superior colliculus
V1[a]	Visual cortex

Note. Most regions were chosen because they have been implicated in motivation, addiction, or locomotor behavior in the literature.
[a] Included as negative controls that were not expected to vary with treatment.

we used the peroxidase method (ABC system; Vector Laboratories, Burlingame, CA) with biotinylated goat anti-rabbit secondary antibodies (Jackson Laboratories, West Grove, PA) and diaminobenzidine as chromogen, enhanced with 0.008% nickel chloride (Sigma, St. Louis, MO).

Image Analysis

The following steps were taken to ensure that Fos-IR was measured consistently between samples:

1. All sections were exposed to diaminobenzidine for exactly 10 min.
2. The background was normalized by automatically adjusting light levels.
3. A threshold level of staining was used to automatically distinguished Fos-positive cells.
4. All slides were coded, and the counting was performed by one individual, who was blind to the experimental conditions.
5. Only Fos-positive nuclei within a specified size range were counted (Guillery, 2002).

We observed no differences in mean size of nuclei for a subsample of mice that differed greatly in Fos-positive cell number (mean nucleus size in DG was 91 pixels squared $\pm$ 35.1 *SD* for an S runner with 143 Fos cells versus 90 pixels squared $\pm$ 28.7 *SD* for an S nonrunner with 55 cells). Fos-positive cells were counted unilaterally, in three alternate sections for each brain region, to obtain an average cell count per brain region for analysis. Microscopic images of the sections were captured by computer with a Zeiss Axiocam high-resolution digital camera (Axiocam; Zeiss, Gottingen, Germany) interfaced to a personal computer running Microsoft Windows. All Fos-positive cells were automatically counted (Zeiss KS300 software) within a box (dimensions are in the legend of Figure 1) placed at the locations shown in Figure 1 (following Paxinos & Franklin, 2001).

Statistical Analysis

Individual mice from each of the eight lines (four S and four C) were studied. Because the lines were separately propagated for 29 generations, mice in a given generation do not represent independent data points (i.e., mice within a line are genetically more similar to one another than mice between lines). Therefore, the individual subjects must be nested within the populations to which they belong (Henderson, 1989, 1997). To satisfy this requirement, line was always entered as a random effect (for theoretical justification of this approach see Pinheiro & Bates, 2000), nested within the fixed effect, line-type (S vs. C) using the Proc Mixed command in SAS (Littell, Milliken, Stroup, & Wolfinger, 1996). The Proc Mixed procedure was chosen because it uses restricted maximum likelihood, which is preferred over least-squares approaches when models include random effects (Littell et al., 1996). To test whether the random effect, line, was significant, a chi-square test statistic was obtained by multiplying -2 by the difference in the restricted log likelihood for the model with versus without the random effect (likelihood ratio test; Pinheiro & Bates, 2000).

Wheel running was analyzed with a one-way nested analysis of variance (ANOVA), with line type entered as a fixed effect and variance among lines estimated separately for C and S mice. Fos counts were analyzed with a two-way nested ANOVA with fixed variables line type, wheel treatment (with or without access to a running wheel), and the interaction between wheel treatment and line type. The *F* statistics and *p* values in Table 2 refer to Type 1, sequential tests of the fixed variables that were entered in this order: wheel treatment, line type, then interaction. Sequential tests can be obtained in Proc Mixed by using the "htype=1" command. Degrees of freedom for testing the line-type effect were always 1 and 6, to reflect the fact that the appropriate experimental unit for testing an effect of line type is the line ($n = 8$), not the individual. Degrees of freedom in the denominator for wheel treatment and the interaction, Wheel Treatment $\times$ Line Type, depended on the number of individual mice; hence, for testing the effect of wheel type and Wheel Treatment $\times$ Line Type interaction, the individual mouse, rather than the line, was considered to be the experimental unit (following a split-plot design; Littell et al., 1996; Pinheiro & Bates, 2000). The number of mice for a given brain region did not always equal 48 because some sections were lost. Fos counts were also analyzed after being raised to an exponent (determined separately for each brain region) in order to stabilize the variance between treatment groups, but these results were qualitatively the same as for untransformed data and so are not shown.

Data were also analyzed by modeling Fos-positive cell number as a function of running distance with separate linear relationships for C and S mice (i.e., terms in the linear model included running distance, line type, Line Type $\times$ Running Distance interaction, and line entered as a random effect). For these analyses, the wheel treatments (blocked or free) were analyzed separately. For blocked mice, total running distance over a 24-hr period, the day before mice were perfused (Day 6) was used as an index of motivation to run on Day 7, although we recognize that it may also reflect variation in physiological abilities for running. For mice with free access to wheels, total distance accumulated until perfusion on Day 7 was used. Wheel-running data were routinely collected at 1 p.m. each day and mice were perfused between 5:30 and 6:30 p.m. (1.5 to 2.5 hours after lights off at 4 p.m.). Thus, running distance accumulated over an approximate 5-hr period prior to perfusion was used in the linear model for free runners. Note that mice normally do not begin running until the dark period begins, however, so most of the running was accumulated during the 1.5 to 2.5 hr

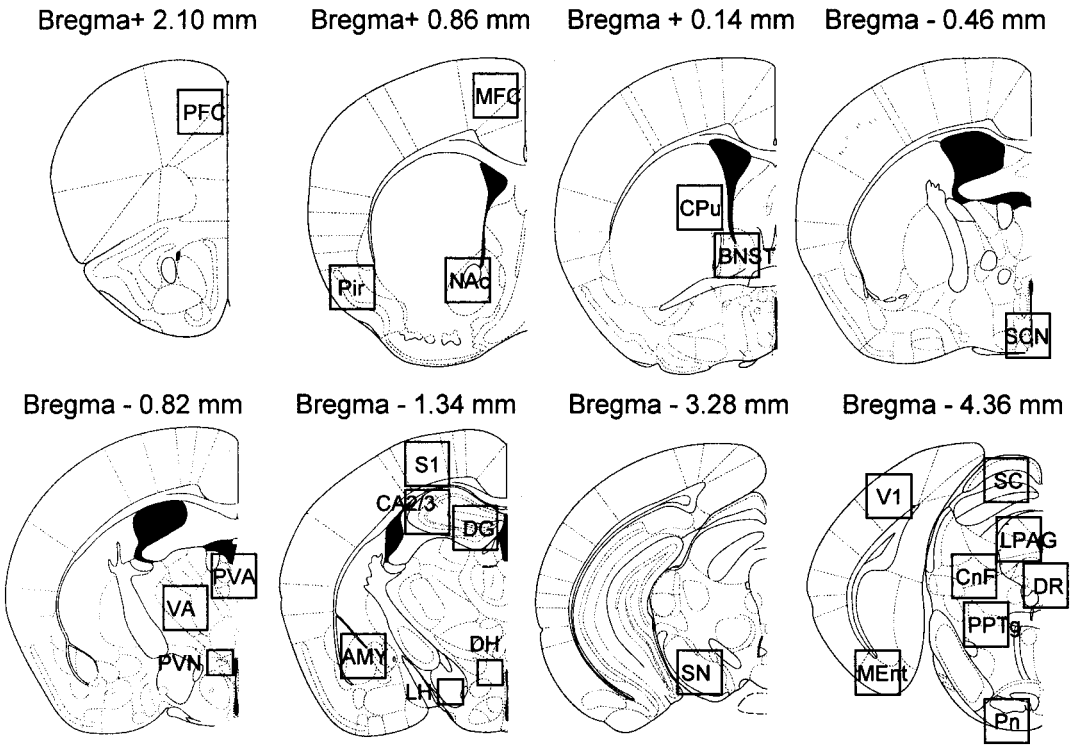


Figure 1. Schematic representation of the brain regions analyzed. The large boxes represent an 870 × 870 μm region; the small boxes, a 435 × 435 μm region, taken under 10× and 20× magnification, respectively. Reprinted from *The Mouse Brain in Stereotaxic Coordinates*, 2nd ed., G. Paxinos and K. Franklin, Figures 13, 24, 30, 38, 42, 58, and 67, Copyright 2001, with permission from Elsevier. PFC = prefrontal cortex; MFC = medial frontal cortex; CPu = caudate–putamen (striatum); Pir = piriform cortex; NAc = nucleus accumbens; BNST = bed nucleus of the stria terminalis; SCN = suprachiasmatic nucleus; S1 = sensory cortex, trunk region; CA2/3 = CA2 and CA3 hippocampal subregions; DG = dentate gyrus; V1 = visual cortex; SC = superior colliculus; LPAG = lateral periaqueductal gray; PVA = paraventricular thalamic nucleus; CnF = cuneiform nucleus; DR = dorsal raphe nucleus; VA = ventral anterior thalamic nucleus; PPTg = pedunculopontine tegmental nucleus; PVN = paraventricular hypothalamic nucleus; AMY = amygdala; LH = lateral hypothalamus; DH = dorsal hypothalamus; SN = substantia nigra; MEnt = medial entorhinal cortex; PN = pontine nucleus.

after lights off prior to sampling. Brains were processed in three batches for the immunohistochemistry, but the batches were always balanced with respect to the treatments, so that batch did not need to be considered in statistical analyses. Body mass was explored as a potential covariate in all analyses, but it was never significant and so was not included in the final analyses. In all instances, $p \leq .05$ was considered statistically significant.

Results

Wheel Running

Twenty-nine generations of selective breeding substantially increased voluntary wheel running (see Figure 2A). We studied male mice (second litters) from Generation 29. During the 6 days when all the mice had free access to the running wheels, mice from the C lines ran on average 3.40 ± 0.60 km/day, whereas S mice ran 9.80 ± 1.33 km/day, $F(1, 6) = 18.8$, $p = .005$. Considering only Days 5 and 6, which is the selection criterion for the breeding experiment (see Swallow et al., 1998), C and S mice ran an average of 4.20 ± 0.71 km/day and 12.3 ± 1.60 km/day, respectively, $F(1, 6) = 21.7$, $p = .004$. C mice ran between 1 and 13 km/day, whereas S mice ran 5 to 31 km/day, and day-to-day variation in running distance was consistent within individual mice (Figure 2B). The large individual variation in voluntary wheel running within both the C and S mice enhances statistical power in an assessment of correlations between levels of voluntary wheel running activity and indicators of neuronal activity (i.e., Fos).

Although S mice ran 2 to 3 times as far as C mice, their pattern of running was similar over the 24-hr cycle (Figure 2C). In both S and C mice, nearly all running occurred during the dark period, with peak running occurring during the first few hours after lights off, consistent with previous observations (Girard et al., 2001; Girard & Garland, 2002).

Comparison of Fos-IR Between Runners and Blocked Mice

As indicated in Table 2, runners had more Fos-positive cells than blocked mice in only 4 of 25 brain regions: DG (Figures 3A, 3B, and 3C), medial entorhinal cortex (MEnt; Figures 3E and 3F),

Table 2
Mean (±SEM) Number of Fos-Positive Cells in 25 Brain Regions of C and S Mice, Blocked From Running or Free to Run on Activity Wheels

Brain region	Blocked		Runner		Two-way ANOVA factors		
	Control	Selected	Control	Selected	Wheel treatment	Line type	Interaction
DG	62 ± 8.2	80 ± 7.0	209 ± 21.5	236 ± 16.6	$F(1, 38) = 108.3$, $p < .0001$	$F(1, 6) = 2.3$, $p = .18$	$F(1, 38) = 0.08$, $p = .78$
CA2/3	78 ± 11.2	106 ± 8.4	123 ± 17.7	151 ± 15.1	$F(1, 38) = 11.9$, $p = .001$	$F(1, 6) = 3.0$, $p = .14$	$F(1, 38) = 0.00$, $p = .98$
BNST	28 ± 2.6	25 ± 2.1	37 ± 3.3	46 ± 6.7	$F(1, 37) = 19.5$, $p < .0001$	$F(1, 6) = 0.0$, $p = 1.0$	$F(1, 37) = 1.4$, $p = .25$
MEnt	112 ± 19.2	110 ± 14.7	182 ± 39.1	286 ± 42.1	$F(1, 28) = 16.7$, $p = .0003$	**$F(1, 6) = 5.8$, $p = .05$**	**$F(1, 28) = 8.3$, $p = .008$**
CPu	227 ± 42.5	369 ± 26.6	103 ± 16.2	123 ± 26.0	$F(1, 38) = 44.4$, $p < .0001$	$F(1, 6) = 4.6$, $p = .08$	**$F(1, 38) = 4.9$, $p = .03$**
PFC	250 ± 46.5	390 ± 31.3	171 ± 22.8	244 ± 34.8	$F(1, 37) = 11.5$, $p < .002$	$F(1, 6) = 5.2$, $p = .06$	$F(1, 37) = 1.0$, $p = .32$
MFC	439 ± 68.7	683 ± 50.4	335 ± 31.4	416 ± 39.2	$F(1, 38) = 16.2$, $p = .0003$	**$F(1, 6) = 5.8$, $p = .05$**	$F(1, 38) = 3.1$, $p = .09$
NAc	70 ± 11.9	109 ± 17.0	41 ± 9.9	40 ± 6.8	$F(1, 36) = 17.7$, $p = .0002$	$F(1, 6) = 1.7$, $p = .25$	$F(1, 36) = 2.9$, $p = .10$
Pir	240 ± 26.1	371 ± 31.4	220 ± 15.3	255 ± 15.8	$F(1, 37) = 11.2$, $p = .002$	$F(1, 6) = 5.3$, $p = .06$	**$F(1, 37) = 5.5$, $p = .03$**
AMY	53 ± 6.7	63 ± 7.6	47 ± 4.8	43 ± 6.8	$F(1, 38) = 4.2$, $p = .05$	$F(1, 6) = 0.1$, $p = .73$	$F(1, 38) = 1.5$, $p = .24$
PVA	285 ± 27.3	351 ± 28.5	234 ± 17.4	261 ± 30.2	$F(1, 38) = 7.4$, $p = .01$	$F(1, 6) = 2.6$, $p = .16$	$F(1, 38) = 0.56$, $p = .46$
VA	158 ± 27.3	220 ± 28.6	106 ± 17.8	146 ± 15.5	$F(1, 38) = 8.6$, $p = .006$	$F(1, 6) = 2.7$, $p = .15$	$F(1, 38) = 0.26$, $p = .62$
S1	428 ± 87.7	877 ± 83.9	293 ± 39.4	311 ± 46.4	$F(1, 38) = 26.7$, $p < .0001$	**$F(1, 6) = 11.8$, $p = .01$**	**$F(1, 38) = 10.1$, $p = .003$**
LH	36 ± 2.2	51 ± 3.3	31 ± 2.7	30 ± 2.0	$F(1, 37) = 24.0$, $p < .0001$	**$F(1, 6) = 6.7$, $p = .04$**	**$F(1, 37) = 9.2$, $p = .004$**
SN	82 ± 14.2	106 ± 12.4	71 ± 7.5	72 ± 5.6	$F(1, 38) = 4.5$, $p = .04$	$F(1, 6) = 1.5$, $p = .27$	$F(1, 38) = 1.3$, $p = .27$
LPAG	156 ± 9.9	161 ± 9.3	143 ± 9.5	132 ± 10.6	$F(1, 36) = 5.1$, $p = .03$	$F(1, 6) = 0.0$, $p = .88$	$F(1, 36) = 0.8$, $p = .37$
DR	290 ± 16.7	318 ± 26.1	207 ± 16.6	162 ± 15.3	$F(1, 36) = 37.7$, $p < .0001$	$F(1, 6) = 0.1$, $p = .74$	$F(1, 36) = 3.4$, $p = .07$
CnF	67 ± 6.3	61 ± 5.4	54 ± 4.5	44 ± 5.9	$F(1, 36) = 7.7$, $p = .009$	$F(1, 6) = 1.5$, $p = .27$	$F(1, 36) = 0.1$, $p = .75$
PPTg	85 ± 8.4	104 ± 9.5	66 ± 7.5	73 ± 8.0	$F(1, 36) = 8.7$, $p = .006$	$F(1, 6) = 2.5$, $p = .17$	$F(1, 36) = 0.4$, $p = .53$
Pn	828 ± 64.0	844 ± 32.1	735 ± 44.6	690 ± 37.8	$F(1, 32) = 12.4$, $p = .001$	$F(1, 6) = 0.4$, $p = .56$	$F(1, 32) = 0.1$, $p = .79$
PVN	97 ± 14.0	148 ± 13.8	90 ± 9.7	131 ± 10.3	$F(1, 38) = 1.1$, $p = .31$	**$F(1, 6) = 14.7$, $p = .009$**	$F(1, 38) = 0.2$, $p = .67$
DH	58 ± 3.8	73 ± 9.0	63 ± 4.2	64 ± 4.8	$F(1, 37) = 0.0$, $p = .95$	$F(1, 6) = 0.7$, $p = .42$	$F(1, 37) = 1.5$, $p = .22$
SCN	340 ± 25.7	409 ± 20.5	356 ± 23.2	366 ± 20.4	$F(1, 29) = 0.3$, $p = .56$	$F(1, 6) = 3.6$, $p = .11$	$F(1, 29) = 1.6$, $p = .21$
SC	217 ± 23.7	272 ± 19.0	264 ± 25.9	322 ± 39.7	$F(1, 36) = 3.4$, $p = .07$	$F(1, 6) = 2.8$, $p = .14$	$F(1, 36) = 0.0$, $p = .96$
V1	478 ± 112.1	601 ± 71.1	650 ± 76.3	739 ± 143.6	$F(1, 31) = 2.3$, $p = .14$	$F(1, 6) = 1.1$, $p = .34$	$F(1, 31) = 0.0$, $p = .87$

Note. Brain regions are organized by effect of wheel access as determined in a two-way nested ANOVA (no covariates were used in these analyses). In the first group of regions (ending with MEnt), runners had significantly higher Fos levels than blocked mice. In the second group (ending with Pn), blocked mice had significantly higher Fos levels than runners, and in the third group no differences between runners and blocked mice occurred. For all three groups, differences between C and S mice are indicated by a significant effect of line type or interaction (boldface). The variance in Fos counts among lines within a line type was significantly greater than zero (i.e., significant effect of replicate line) for Pir, $\chi^2(1, N = 47) = 4.4$, $p = .04$; BNST, $\chi^2(1, N = 47) = 4.7$, $p = .03$; and DH, $\chi^2(1, N = 47) = 9.1$, $p = .003$, $n = 12$ in each group.

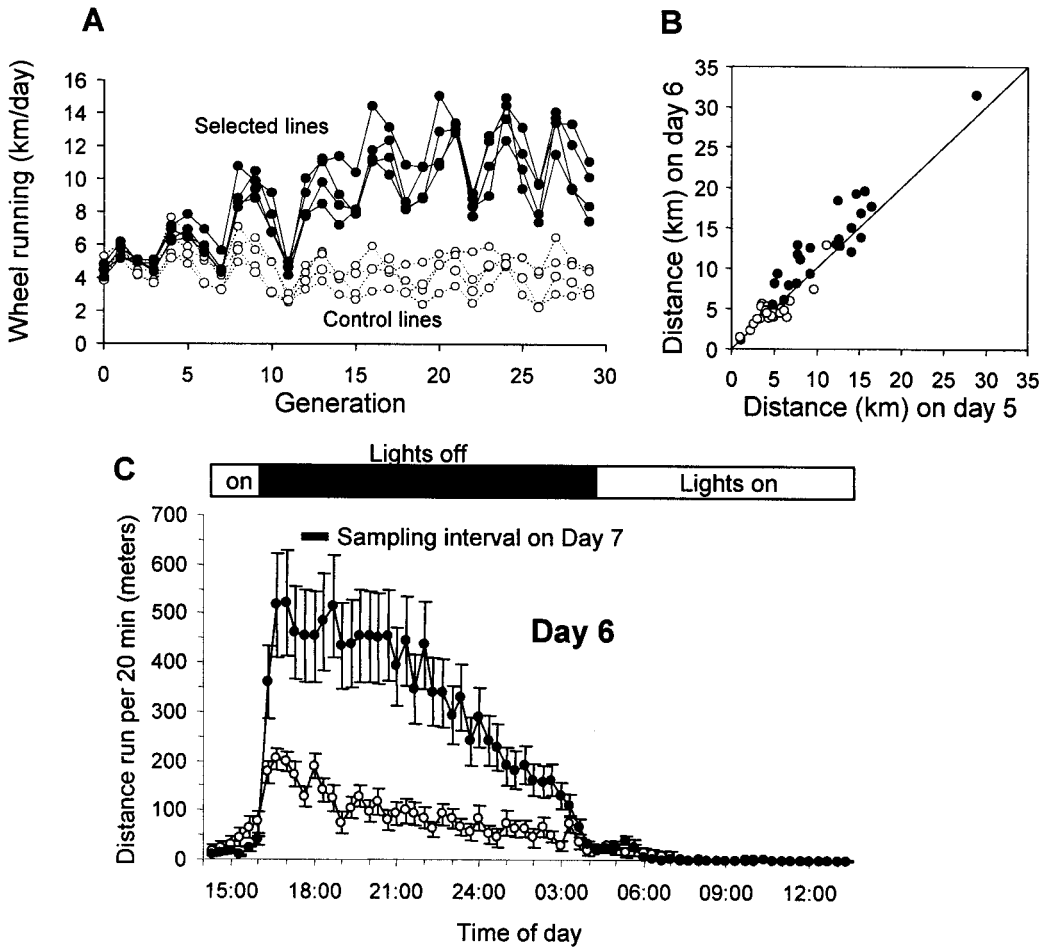


Figure 2. A: Twenty-nine generations of selective breeding has produced four replicate lines of mice (S mice) that display increased voluntary wheel running as compared with four random-bred control lines (C mice). Mean distance (kilometers/day) run by males on Days 5 and 6 of a 6-day test are shown for each line across generations, from the beginning of the selective breeding through Generation 29. Note that the differential has remained approximately constant since Generation 16 (see also Garland, 2003). B: Running distance was consistent within individual mice across days, as demonstrated by the close association between distance run on Day 5 and distance run on Day 6 for the mice used in the Fos experiments (second litters from Generation 29). The mouse that ran an exceptional 31 km/day on Day 6 is one of the highest recordings ever for a male mouse from selected Line 6 (J. S. Rhodes, personal observations). The one-to-one line is shown. C: The pattern of running over the 24-hr cycle is shown for S and C mice on Day 6 of the continuous wheel access period, as mean (± *SEM*) distance run per 20 min (some *SEM*s are too small for error bars to be visible). Peak running occurred in the first few hours after lights off for both S and C mice, consistent with previous observations (Girard et al., 2001; Girard & Garland, 2002). The time interval when mice were sampled on Day 7 for Fos immunoreactivity is shown to illustrate when mice were sampled relative to the normal pattern of running. At this time, Fos immunoreactivity would indirectly reflect neuronal activity during the previous 2 hr, when both S and C mice normally display peak activity on running wheels.

CA2/3, and bed nucleus of the stria terminalis (BNST). In contrast, 16 of 25 brain regions showed higher Fos in the blocked mice as compared to runners: CPu (Figures 4A and 4B); PFC (Figures 4E and 4F); MFC; NAc; amygdala (AMY); Pir; paraventricular hypothalamic nucleus (PVA); ventral anterior thalamic nucleus (VA); sensory cortex, trunk region (S1); LH; substantia nigra (SN); LPAG; dorsal raphe nucleus (DR); CnF; PPTg; and Pn. The only regions unaffected by the wheel treatment were the SCN, PVN, dorsal hypothalamus (DH), superior colliculus (SC), and V1 (Table 2). For SCN and V1, this matched expectations because these regions were included as negative controls.

Comparison of Fos-IR Between S and C Mice

Fos-IR was compared between S and C mice within the runner treatment and also within the blocked treatment. C mice did not have significantly higher levels of Fos-IR than S mice under any condition. Among the mice that were allowed to run the day they

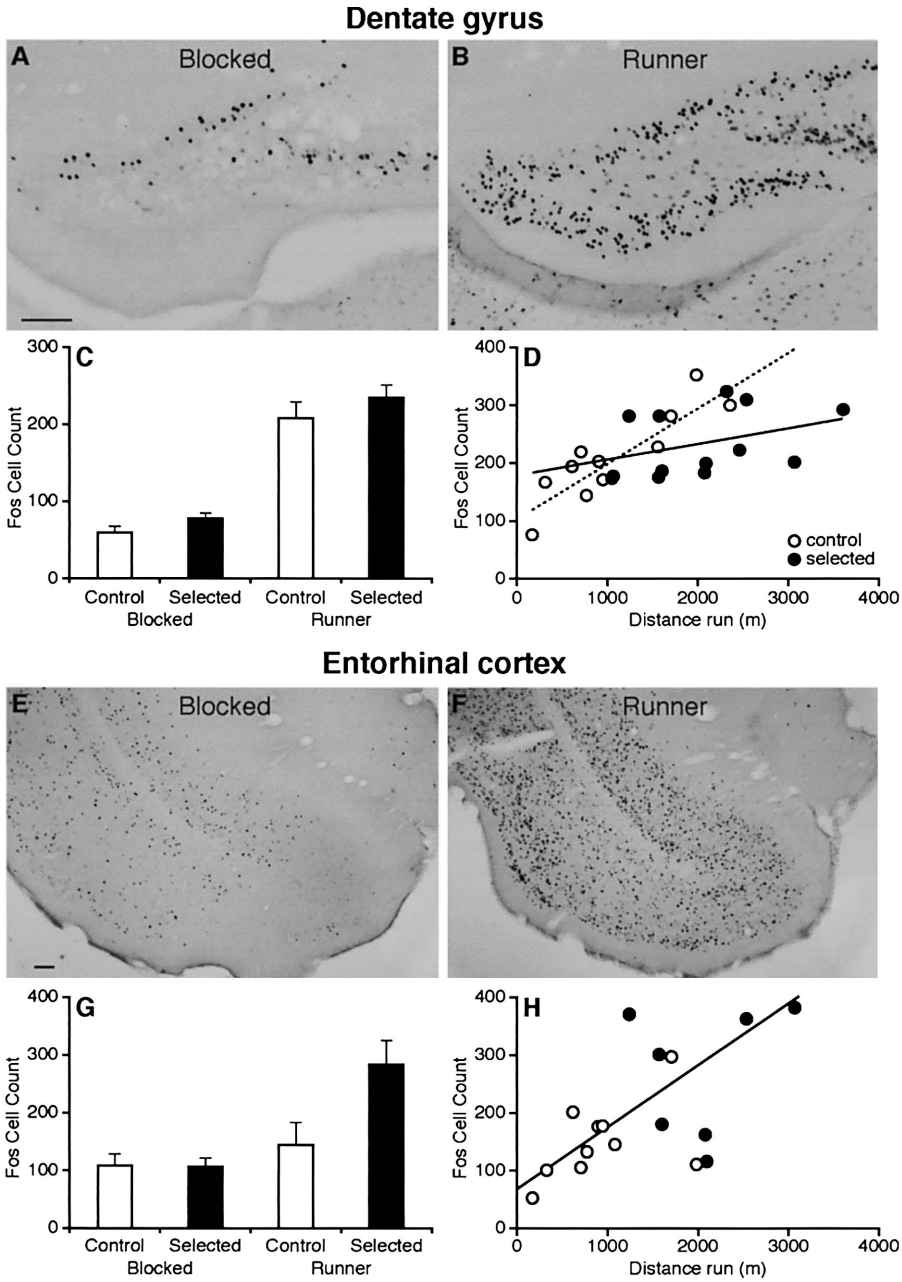
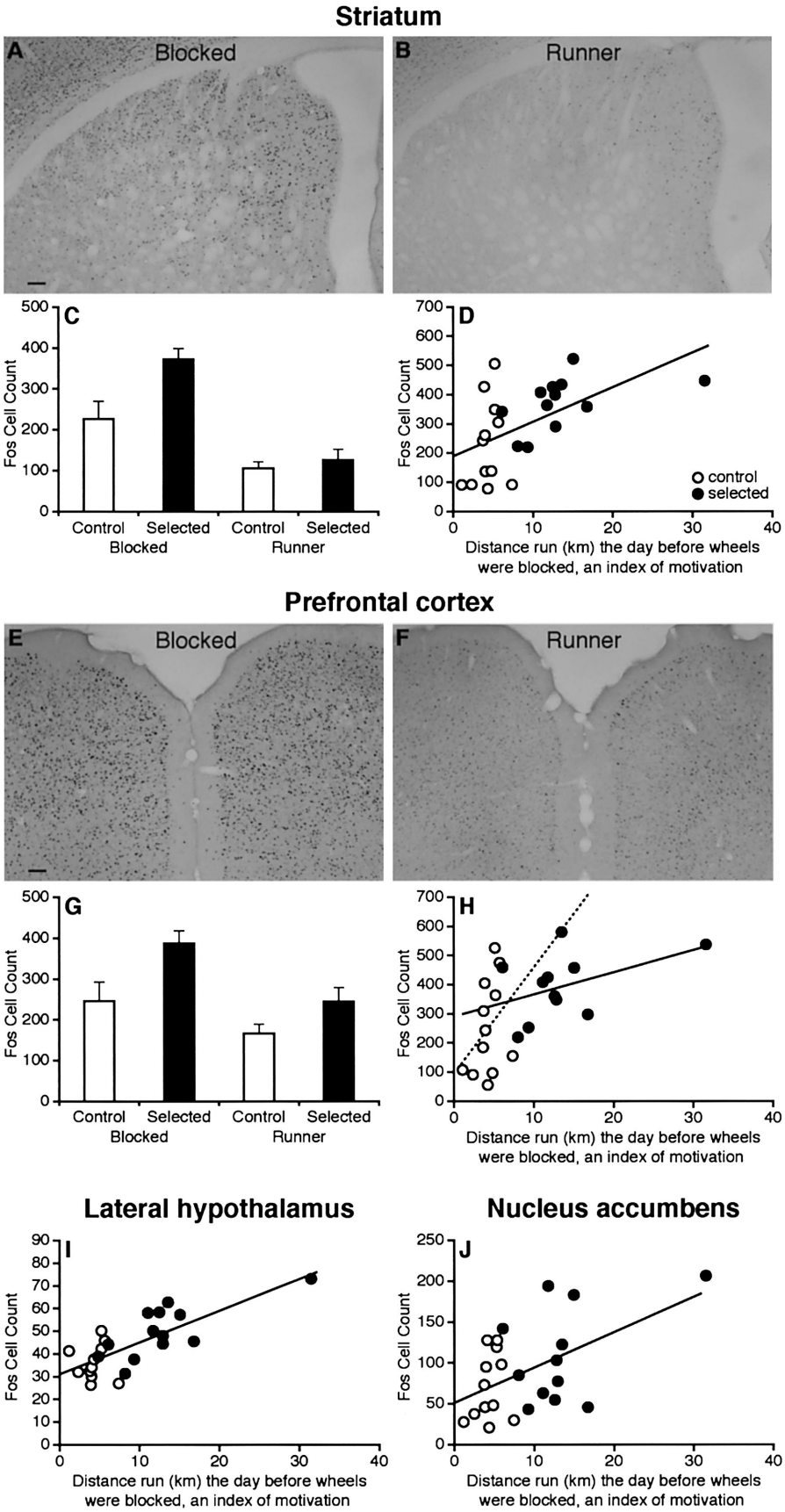


Figure 3. Running induced Fos in the dentate gyrus (DG) and entorhinal cortex (MEnt). Representative section through DG of S mice, blocked from running (A) and free to run (B). Bar graph (C), displaying mean (± *SEM*) number of Fos-positive cells in DG of mice blocked from running versus free to run, from C and S lines. Scatter plot (D), showing a significantly steeper linear relationship between distance run and number of Fos-positive cells (interaction between distance run and line type, $p = .03$) in DG of C mice than S mice with free access to running wheels. Legends are the same for MEnt (Panels E, F, G, and H), except that in Panel H, S and C data points fell along the same linear relationship (i.e., no line type or interaction effects). Scale bars = 200 μm (A, B) and 500 μm (E, F). S = mice selectively bred for increased voluntary wheel running; C = control mice.

were sampled, Fos-IR was higher in S than C mice in the entorhinal cortex (MEnt) and PVN, as indicated by significant effects of line type and Wheel Treatment × Line Type interaction and inspection of the means in Table 2. Post hoc Tukey's comparisons of S versus C runners were not significant for MEnt ($p = .11$) nor for PVN ($p = .09$).

Among the mice that were prevented from running the day they were sampled, Fos-IR was higher in S than C mice in CPu (Figure 4C), MFC, S1, LH, Pir, and PVN, as indicated by significant effects of line type and Wheel Treatment × Line Type interaction and inspection of the means in Table 2. Post hoc Tukey's comparisons of S versus C mice within the blocked treatment were


Striatum
A
Blocked
B
Runner
C
Fos Cell Count
Control
Selected
Control
Selected
Blocked
Runner
D
Fos Cell Count
control
selected
Distance run (km) the day before wheels were blocked, an index of motivation
Prefrontal cortex
E
Blocked
F
Runner
G
Fos Cell Count
Control
Selected
Control
Selected
Blocked
Runner
H
Fos Cell Count
Distance run (km) the day before wheels were blocked, an index of motivation
Lateral hypothalamus
I
Fos Cell Count
Distance run (km) the day before wheels were blocked, an index of motivation
Nucleus accumbens
J
Fos Cell Count
Distance run (km) the day before wheels were blocked, an index of motivation

significant for CPu ($p = .02$), MFC ($p = .02$), S1 ($p = .02$), LH ($p = .002$), Pir ($p = .02$), and PVN ($p = .02$).

Correlation Between Fos-IR and Distance Run

Considering both S and C runners together, the distance run (accumulated over an approximate 5-hr period prior to sampling; see the *Statistical Analysis* section) was positively correlated with Fos-IR in DG, $F(1, 14) = 14.8$, $p = .002$ (Figure 3D); MEnt, $F(1, 12) = 9.9$, $p = .009$ (Figure 3H); SC, $F(1, 9) = 11.8$, $p = .008$; VA, $F(1, 14) = 5.8$, $p = .03$, and marginally uncorrelated in Pn, $F(1, 10) = 4.7$, $p = .06$, and SN, $F(1, 14) = 4.1$, $p = .06$. In DG, the linear relationship was steeper for C than S mice, as indicated by a significant interaction between line type and distance run, $F(1, 14) = 6.2$, $p = .03$; the main effect of line type was not significant $F(1, 6) = 1.5$, $p = .27$ (Figure 3D). Considering only C runners, Fos-IR in DG was strongly correlated with distance run, $F(1, 7) = 77.2$, $p < .0001$. C mice that ran 2,000 m prior to sampling had approximately three times the number of Fos-positive cells in DG as C mice that ran 200 m (Figure 3D). In S runners considered alone, however, Fos-IR in DG was not significantly correlated with distance run, $F(1, 7) = 1.3$, $p = .29$. S mice that ran 3,000 m prior to sampling had approximately the same number of Fos-positive cells in DG as S mice that ran 1,000 m (Figure 3D). These results suggest that there is a limit to the number of DG cells that can be activated in association with wheel running, and that most S mice reach this limit by virtue of their high levels of wheel running. In the other regions, the linear relationship was similar for C and S mice (i.e., the main effect of line type and the interaction were not significant; e.g., Figure 3H).

Correlation Between Fos-IR and an Index of Motivation to Run

Because running distance was consistent within individual mice from day to day (Figure 2B), running distance the day before wheels were blocked (Day 6) was considered an index of motivation for running the day mice were prevented from running (Day 7). Motivation for running, considering only mice that were blocked from access to wheels, was positively correlated with Fos-IR in LH, $F(1, 14) = 26.7$, $p < .0001$ (Figure 4I); S1, $F(1, 14) = 13.3$, $p = .003$; CPu, $F(1, 14) = 5.6$, $p = .03$ (Figure 4D); PFC, $F(1, 14) = 6.5$, $p = .02$ (Figure 4H); MFC, $F(1, 14) = 9.4$, $p = .008$; NAc, $F(1, 14) = 7.5$, $p = .02$ (Figure 4J); and Pir, $F(1, 14) = 8.7$, $p = .01$. In PFC (Figure 4D) and MFC, the linear relationship was steeper for C than S mice, as indicated by a significant interaction between line type and distance run for PFC, $F(1, 14) = 7.0$, $p = .02$, and MFC, $F(1, 14) = 4.8$, $p = .05$; the main effect of line type was not significant for PFC, $F(1, 6) = 0.1$, $p = .76$, nor for MFC, $F(1, 6) = 0.1$, $p = .72$. These results suggest that there is a limit to the number of cells in PFC and MFC that can be activated when running is blocked, and that S mice reach this limit by virtue of their higher "dependence" on wheel running. In the other regions, data points for C and S mice fell along the same line (i.e., there was no significant main effect of line-type or interaction between line-type and distance run the previous day). Second-degree polynomial curves did not fit the data shown in Figures 4D, 4H, 4I, and 4J significantly better than did simple linear relationships (as judged by a marginal test of significance of the polynomial coefficient).

Figure 4 (*opposite*). "Motivation" for running induced Fos in the striatum (caudate–putamen; CPu), prefrontal cortex (PFC), lateral hypothalamus (LH), and nucleus accumbens (NAc). Representative section through the CPu of S mice, blocked from running (A) and free to run (B). Bar graph (C), displaying mean (± *SEM*) number of Fos-positive cells in the CPu of mice blocked from running versus free to run, from C and S lines. Scatter plot (D) illustrating an apparently linear relationship between distance run the day before wheels were blocked (an index of "motivation" to run) and Fos-positive cells in the CPu of blocked mice from C lines and S lines. Legends are the same for the PFC (Panels E, F, G, H), except that the scatter plot Panel H shows a significantly steeper linear relationship (interaction, $p = .02$) for C mice (open circles) versus S mice (filled circles). Panels I and J display data for the LH and NAc in the same form as (D). Scale bars = 500 μm (Panels A, B, E, and F). S = mice selectively bred for increased voluntary wheel running; C = control mice.

Discussion

Recently, it has been proposed that wheel running is naturally rewarding and addictive (Belke, 1996; Belke & Belliveau, 2001; Iversen, 1993; Lett et al., 2000; Nestler et al., 2001; Werme et al., 2002). This study provides a unique perspective by examining brain activity in mice that had been subject to 29 generations of selective breeding for increased voluntary wheel-running exercise. Both our approach of using selectively bred lines of mice and our experimental design, which includes voluntary runners along with mice denied their regular exercise routine, are unique. Here we provide the first evidence that brain regions can evolve, in response to genetic selection, to increase motivation for wheel running. Further, we provide the first evidence that brain activity in the hippocampus may ultimately limit voluntary exercise in high-running S mice.

The S mice that were bred for increased voluntary wheel running are highly motivated to run on wheels, and when they are denied access to their wheels, they show high levels of brain activity, relative to unselected C mice, in brain regions that have been implicated in arousal (LH; Espana, Baldo, Kelley, & Berridge, 2001), natural reward (MFC; Schroeder et al., 2001), and initiation of locomotion (CPu; Jordan, 1998). When the mice are allowed to run, these same regions show relatively low levels of activity, no statistical difference from C mice, and no correlation with running distance. Thus, we suggest that LH, MFC, and CPu play a role in motivation for wheel running by reflecting increased appetitive value of running, or enhanced frustration, anger, anxiety, or stress when running is denied. Other regions, such as DG, strongly reflected differential levels of running among C mice ("normal" mice), which is consistent with a possible role for DG in the control of the intensity of the running itself.

Putative Brain Regions Associated With Variation in the Exercise Itself

Our data are consistent with the hypothesis that Fos activity in the hippocampus plays an important role in exercise, as suggested by Oladehin and Waters (2001), who studied effects of forced treadmill exercise on Fos-IR in the hippocampus of rats. The

strong correlation ($p < .0001$; Figure 3D) between amount of voluntary exercise (distance run) and Fos in the DG of C runners could reflect a passive role of the hippocampus in receiving sensory input from running, or an active role in modulating the motor behavior (Bardgett & Henry, 1999; W. L. McFarland, Teitelbaum, & Hedges, 1975; McNaughton, Barnes, & O'Keefe, 1983; Morris & Hagan, 1983; Oddie & Bland, 1998; Oladehin & Waters, 2001; Slawinska & Kasicki, 1998; Vanderwolf, 1969).

The strong correlation between distance run and number of Fos cells in the DG of C runners was lost in S runners (Figure 3D). One interpretation of this result is that there is a limit to the number of DG cells that can be activated in association with wheel running, which most or all S mice reach by virtue of their high amount of exercise. This is the first evidence of which we are aware that the number of neurons activated in association with a specific behavior can reach an upper limit. The implication of this finding is noteworthy if DG plays a role in modulating the motor behavior. In this case, one would predict that the more neurons an animal can activate in DG, the more intensely it can run, and that a limit in number of DG neurons would limit expression of running. It is interesting that, in a recent study we found more granule neurons and greater running-induced neurogenesis in S than C mice in DG (Rhodes et al., 2003). Moreover, neurogenesis showed a pattern of association with running distance similar to that of Fos-IR. A strong correlation occurred between running distance and neurogenesis in DG of C mice, but the relationship reached a plateau in S mice (Rhodes et al., 2003). It is unlikely that patterns of Fos-IR in DG simply reflected early stages of neurogenesis because Fos-positive cells occurred throughout the granule cell layer (Figures 3A and 3B), whereas neurogenesis occurs only at the inside layer (adjacent to the hilus) of DG. Taken together, these data suggest that activation of DG may limit wheel-running behavior, and that neurogenesis can increase this capacity, but that a plateau exists for this process. This is important because it might explain why continued selective breeding has not produced greater levels of wheel running in the S lines since approximately Generation 16 (Figure 2A; Garland, 2003).

Arguments against a role for the hippocampus in motor function are based on evidence that its destruction does not prevent locomotion (as reviewed in Oddie & Bland, 1998). However, destruction of the hippocampus *does* change qualitative aspects of movement execution, such as the intensity at which a motor act can be carried out (Oddie & Bland, 1998). For example, although hippocampal lesions do not prevent rats from jumping (Myhrer, 1975), they reduce the height to which rats are capable of jumping (Oddie & Bland, 1998). Moreover, the frequency of theta waves in the hippocampus increases immediately preceding a jump ($\sim$ 100 ms), and the length of the period of the theta wave coinciding with take-off is strongly and inversely correlated with the height, velocity, and peak force parameters of the jump (Morris & Hagan, 1983). Theta activity in the rat hippocampus is necessary to induce spontaneous wheel-running behavior via electrical stimulation of the posterior hypothalamus (Oddie & Bland, 1998), and the frequency of this hippocampal theta (adjusted by the intensity of electrical stimulation) is closely correlated with wheel-running speed (Slawinska & Kasicki, 1998). Thus, although rodents may be able to move without a hippocampus, they cannot produce intense movements typically associated with some types of locomotor exercise (e.g., high jumps or relatively fast wheel running). Given the potential role of DG in regulating the intensity of wheel running, it is possible that an upper limit of cells activated during running in DG of S mice limits the intensity of wheel-running exercise. However, whether the number of Fos-positive cells in DG during running reflects passive sensory information from running or a role of the hippocampus in causing running will need to be determined through additional experiments.

The mesencephalic locomotor region (MLR), including CnF, LPAG, PPTg, and Pn, has been hypothesized to directly activate corticospinal cells to initiate locomotion (Jordan, 1998). Therefore, it is somewhat surprising that we did not find any differences in activation of MLR between S and C runners, nor any correlation between distance run and Fos-IR in MLR (although distance run was marginally uncorrelated with Fos-IR in Pn, see the *Correlation Between Fos-IR and Distance Run* section). It is possible that activation of the MLR reflects qualitative, but not quantitative, differences in locomotor activity. Thus, had we included a control treatment of no preexposure to wheels, we might have seen elevated Fos in the MLR (see *Methodological Considerations* below). Regardless, the objective of the study was to identify brain regions that play a role in the differential wheel running of S and C mice, and MLR did not appear to function in this respect.

Putative Brain Regions Associated With Variation in Motivation for Exercise

The S mice represent a powerful model to study the neural basis of motivation for exercise because they have been bred for many generations to display increased *voluntary* wheel running. When denied their regular exercise routine, the S mice display high levels of brain activity, relative to C mice, in brain regions involved in natural and drug reward (e.g., MFC, CPu, Table 3; Kelley & Berridge, 2002). This supports the interpretation that S mice may have been selected to become "addicted" to wheel running. The MFC has been implicated in drug reward in rats by results showing that this region is activated when nicotine or morphine is withheld (Schroeder et al., 2001, 2003). The CPu is better known for its role in voluntary movement (N. R. McFarland & Haber, 2000; Ouchi et al., 2002; Saper, 1996), but also plays a role in reward (Kelley & Berridge, 2002). However, other regions that showed a greater elevation of Fos-IR in S as compared with C mice within the blocked treatment are not typically associated with brain reward or locomotor circuitry (e.g., Pir, S1). These regions might have reflected increased frustration, anger, or stress resulting from denial of wheel running in S versus C mice. Alcohol withdrawal increased Fos-IR in the Pir and cerebral cortex of rats (Wilce, Beckmann, Shanley, & Matsumoto, 1994). Thus, taken together, the brain regions implicated in motivation for running in Table 3 support the hypothesis that wheel running is rewarding and addictive (Belke, 1996; Belke & Belliveau, 2001; Iversen, 1993; Lett et al., 2000; Nestler et al., 2001; Werme et al., 2002). The possible implication of these results for humans is that, for some individuals, chronic high levels of exercise (e.g., running) may be a form of addiction.

We interpreted differences between S and C mice denied wheel access as reflecting differential motivation for running, appetitive value or withdrawal. However, acute effects of physical activity itself could stimulate neurons and be responsible for differences in Fos-IR between S and C blocked mice, because S mice may be

Table 3
A Summary of the Major Findings of This Study

Motivation regions	Exercise regions
CPu—Caudate-putamen complex	DG—Dentate gyrus of hippocampus
PFC—Prefrontal cortex	MEnt—Medial entorhinal cortex
MFC—Medial frontal cortex	
NAc—Nucleus accumbens	
Pir—Piriform cortex	
S1—Sensory cortex, trunk region	
LH—Lateral hypothalamus	

Note. Regions on the left-hand side are putative sites that evolved in mice selected for high voluntary wheel running (S mice) to increase motivation for exercise. In these motivation regions, Fos was either higher in S than in control (C) mice when mice were blocked from running, or Fos was correlated with amount of exercise the previous day in blocked mice. Regions on the right-hand side are putative sites that control the intensity of the locomotor behavior itself. In these exercise regions, Fos immunoreactivity was either strongly associated with distance run, or was higher in S than in C mice after running.

more active than C mice even in cages without wheels (Rhodes et al., 2001). Results for the separate group of mice sampled after running argue against the latter possibility, though, because if acute effects of physical activity account for the differences between S and C blocked mice, then we would expect to find a correlation between distance run and Fos-IR in the runners, but we found no such correlation for the "motivation regions" listed in Table 3.

Another strategy we used to identify brain regions that may be involved in motivation for exercise was to examine the correlation between Fos and distance run on the day before wheels were blocked in exercise-denied mice. Distance run the previous day serves as an index of motivation in exercise-denied mice, because it accurately predicts how much such a mouse would want to run if it could (i.e., wheel running is a highly repeatable behavior, Figure 2B; see also Friedman, Garland, & Dohm, 1992). The correlation analyses support and extend (by including PFC and NAc) the hypothesis that motivation for wheel running is associated with elevated Fos in brain regions implicated in natural and drug rewards (Figure 4). The NAc and PFC (Table 3) are widely considered to be important regions in motivation for natural rewards (Kelley & Berridge, 2002; Miller, 2000), including voluntary wheel running (Nestler et al., 2001; Werme et al., 2002), and are also recognized as part of the pathways initiating locomotion (Brudzynski & Gibson, 1997; Jordan, 1998).

Among the brain regions implicated in motivation for exercise (Table 3), LH stands out as potentially playing a pivotal role, because this region showed the strongest correlation between Fos and distance run the day before perfusion in exercise-denied mice ($p < .0001$; Figure 4I). LH has previously been implicated in both treadmill running (Iwamoto et al., 1996) and the initiation of locomotion (Jordan, 1998). Electrical stimulation of LH induces spontaneous locomotion in both anesthetized (Sinnamon & Stopford, 1987) and awake rats (Whishaw, Bland, & Vanderwolf, 1972). LH plays an important role in arousal via the protein neurotransmitter orexin. Orexin cell bodies are found only in LH and adjacent regions and send projections to the basal forebrain (including NAc; Espana et al., 2001) and the ventral midbrain (Fadel & Deutch, 2002) of rats, and thereby can affect brain-reward or motor circuitry.

One important factor that might have affected neuronal activity throughout the brain in the mice blocked from accessing their running wheels is stress. We are not aware of any studies that have explicitly tested the hypothesis that blocking wheel access induces a stress response. Wheel running itself elevates plasma corticosterone relative to nonrunning sedentary mice that have never been exposed to wheels (Girard & Garland, 2002). Our data suggest that blocking wheel access is no more stressful than wheel-running because the PVN, which produces corticotropin releasing hormone to initiate the stress response at the level of the hypothalamic–pituitary–adrenal (HPA) axis (Honkaniemi et al., 1992; Imaki, Shibasaki, Hotta, & Demura, 1992; Zhu et al., 2001), displayed equivalent Fos activation in the runners as in the blocked mice (see Table 2, effect of wheel type $p = 0.31$). Although blocking wheel access did not increase Fos-IR in the PVN of S or C mice relative to runners, PVN was the only region where Fos was higher in S than C mice under both conditions, blocked and runner. This finding is consistent with the finding of higher circulating levels of corticosterone in S than C female mice, regardless of whether mice were housed with or without wheels (Girard & Garland, 2002). Thus, it is possible that differences in the HPA axis underlie the differential wheel running and differential patterns of Fos activation throughout the brains of S versus C mice. However, whether the PVN plays a role in the increased motivation for running in S mice or is activated by acute effects of physical activity cannot be determined from the present study.

Possible Connections Between Dopamine, High Motivation for Wheel Running, and ADHD

Previous pharmacological studies suggest that dopamine function is altered in S mice (Rhodes et al., 2001; Rhodes & Garland, 2003), and dopamine is an important modulator of neuronal activity in several of the brain regions implicated in motivation for running in Table 3. Thus, we speculate that altered dopamine function may have been responsible for differential activation of such regions as PFC, NAc, MFC, and CPu when running was denied.

We have argued previously (Rhodes et al., 2001; Rhodes & Garland, 2003) that the S mice represent a useful model of attention-deficit/hyperactivity disorder (ADHD). Functional imaging studies report that metabolism in the frontal cortex and striatum is reduced in ADHD (as reviewed in Castellanos, 2001). We found increased Fos in these same regions in S versus C mice that were

denied exercise. Whether Fos reflects an increase or a decrease in metabolism (as could be measured by functional imaging) in the mice is not known. Nonetheless, it is intriguing that similar brain regions are implicated in the hyperactivity of S mice and in ADHD.

Methodological Considerations

The aim of this study was to identify brain regions that are differentially activated in the S versus C mice under two conditions: blocked from wheel access to which they had become accustomed for 6 days and continued wheel access (runner). We did not include a treatment in which mice were never exposed to wheels, but our design does allow for the examination of the acute effects of wheel running. For example, runners (both S and C) had 3-fold higher levels of Fos-IR than blocked mice in DG. Because Fos-IR peaks approximately 2 hr after a stimulus and decays in a predictable manner (Nestler et al., 2001), the elevation of Fos in running mice represents increased neuronal activity relative to the mice not allowed to run. Examination of the correlation between Fos-IR and distance run confirms the positive association between these two measures.

Acute administration of abused drugs transiently increases Fos in brain regions involved in reward such as NAc (Werme et al., 2002), but in this study, NAc Fos levels were lower in the runners than in the blocked mice, even though substantial evidence suggests that wheel running is rewarding (Belke, 1996; Belke & Belliveau, 2001; Iversen, 1993; Lett et al., 2000; Nestler et al., 2001; Werme et al., 2002). This difference might be attributable to the fact that we measured mice after 6 days of wheel access because after repeated drug treatment, induction of immediate early gene products such as Fos declines (Nestler et al., 2001; Werme et al., 2002). In addition, NAc activity might have been elevated in the blocked mice relative to the runners, in association with withdrawal, motivation, or in response to the novel condition of being denied access to the running wheel.

This study compares Fos-IR between S and C mice at the time of normal selection of breeders, when mice are normally running at peak levels (Garland, 2003). Phenotypic differences between S and C mice at any time are genetically based and can arise as a cause, a consequence, or a pleiotropic effect of the genes that increase running (linkage disequilibrium is also possible, though unlikely). The simplest scenario in the current study is that a difference in Fos-IR between S and C mice reflects the role of a brain region in causing the variation in running. For example, LH may have evolved in S mice to become excitable when the mouse is not running to arouse the ventral midbrain and forebrain to motivate wheel running. Another scenario is that a brain region is activated in response to the physical activity itself. For example, it is possible that DG becomes activated in proportion to amount of running because DG receives sensory information from running. Yet another possibility is that the genes responsible for the variation in running have a pleiotropic effect on a brain region not directly involved in running or reward circuitry. For example, altered function of dopamine in MFC might have evolved to motivate running in S mice, but the altered dopamine function might be nonspecific and also affect the PVN, even though the PVN plays no role in motivating wheel running.

Any of the scenarios described above could explain a difference in Fos-IR between S and C runners. When interpreting a difference between S and C blocked mice, an acute effect of running can be excluded, because running was prevented and the marker of neuronal activity that was used reflects only short-term changes in brain activity. It is still possible that the gradual accumulation of effects of running over the 6 days altered brain activity such that specific regions became differentially activated in mice in the blocked condition. A separate study examining Fos changes with running experience over time could help to address the long-term effects of exposure to running wheels. Because S mice may be more active than C mice in cages without wheels (Rhodes et al., 2001), it would be difficult to exclude a possible cumulative effect of activity, even if a group not exposed to wheels had been included in the experimental design.

The limitations of using Fos as a marker of brain activity have been reviewed elsewhere (Dragunow & Faull, 1989; Harris, 1998). We have followed steps to avoid the pitfalls as described in Dragunow and Faull (1989) and Harris (1998). For example, because the time-course for inducing Fos can vary with brain region, we did not compare Fos between brain regions. We also acknowledge that not all neurons produce Fos in response to stimulation (Dragunow & Faull, 1989), and in other neurons, only a very strong stimulus leads to Fos activation, leaving open the possibility for false negatives. In addition, Fos can be expressed without neuronal activation (e.g., in response to hormones or growth factors), leading to false positives. Moreover, an elevation in Fos might reflect an increase or a decrease in brain activity, because signal transduction cascades involving Fos could be excitatory or inhibitory.

Summary

To the best of our knowledge, this is the first study designed to identify short-term changes in brain activity associated with variation in *voluntary* exercise in rodents. To achieve this aim, mice were bred to display increased voluntary wheel running, and Fos-IR was compared between the selectively bred (S) and unselected (C) mice. The seven brain regions that emerged as potentially being responsible for the variation in running between S and C mice (motivation) are listed in Table 3. We recognize that differences between S and C mice could reflect causes of increased running, effects of increased physical activity, or complex pleiotropic influences, all of which are genetically based. We suggest that DG may play an important role in controlling the intensity of wheel-running behavior (see also Bardgett & Henry, 1999; W. L. McFarland et al., 1975; McNaughton et al., 1983; Morris & Hagan, 1983; Oddie & Bland, 1998; Oladehin & Waters, 2001; Vanderwolf, 1969), on the basis of the close association between running distance and Fos-IR in C mice at this region. We suggest that brain regions including LH, MFC, and CPu play a role in the motivation to run, because Fos-IR was higher in these regions in S than in C mice when both were denied exercise. It is possible that LH, MFC, and CPu evolved, in response to selective breeding, to increase the appetitive value of voluntary wheel running, whereas DG did not evolve but merely functions in the exercise itself. If DG functions in controlling the intensity of running, then these results suggest that activation in this region may ultimately limit exercise capacity

because exercise-associated neuronal activity in DG reached a plateau in S runners.

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