The Fe:ferritin molecule ratio was kept at 36:1. The reaction of apoferritin with Fe^{2+} and O_2 was quenched at 25 ms when the peroxodiferric intermediate had accumulated maximally, and at 1 s when the intermediate had decayed.

10. The experiment was conducted at 3.0 GeV and 50 to 100 mA, with the use of a Si(220) double-crystal monochromator that was detuned 50%, with an Fe foil internal calibration (first inflection point at 7111.2 eV). We used a 13-element Ge solid-state fluorescence detector (incident count rate <60 kHz per channel, about 3-kHz Fe Kα fluorescence counts) at 10 K. The step size was 10 eV in the pre-edge region (6880 to 7080 eV), 0.35 eV in the edge region (7080 to 7140 eV), and 0.05 $Å^{-1}$ in the EXAFS region (2 to 13 $Å^{-1}$). Integration times ranged from 1 s in the pre-edge region to 20 s at k = 13 $Å^{-1}$, where k is the photoelectron wavevector.
11. XANES data were normalized to tabulated x-ray cross sections, EXAFS data were extracted by subtracting a first-order pre-edge polynomial, and then a three-region cubic spline was fitted to the EXAFS. Data converted to k space with E_0 = 7130 eV and weighted by k^3. k space data (1.5 to 11.9, 12.4, and 12.7 $Å^{-1}$ for $-O_2$, $+O_2{}^{25\ ms}$, and $+O_2{}^{1\ s}$ samples, respectively) were Fourier transformed to give the R space data in Fig. 2.
12. Bond lengths, coordination numbers, and Debye-Waller factors determined from fitting Fourier-filtered data (R = 1.0 to 3.2, 1.0 to 3.3, and 1.0 to 3.6 Å for the $-O_2$, $+O_2{}^{25\ ms}$, and $+O_2{}^{1\ s}$ samples, respectively) with the use of theoretical amplitude and phase functions calculated with FEFF v.6.01. [J. J. Rehr, J. Mustre de Leon, S. I. Zabinsky, R. C. Albers, *J. Am. Chem. Soc.* **113**, 5135 (1991)], with the scale factor (0.9) and ΔE_0 (10 eV) calibrated from fits to data for crystallographically characterized models. Coordination numbers were fixed at reasonable values, and only R and σ were treated as freely variable parameters. Identical metric parameters were obtained for fits to filtered and unfiltered data. A weighted goodness of fit $F' = F^2/\nu = F^2/(N_{idp} - N_{var})$ was used to evaluate the quality of the fit [P. J. Riggs-Gelasco, T. L. Stemmler, J. E. Penner-Hahn, *Coord. Chem. Rev.* **144**, 245 (1995)], where F is the root mean square difference between the fit and the data, N_{idp} is the number of independent data points, and N_{var} is the number of variable parameters.
13. S. M. Kauzlarich *et al.*, *Inorg. Chem.* **25**, 2781 (1986); J. G. DeWitt *et al.*, *J. Am. Chem. Soc.* **113**, 9219 (1991).
14. A. L. Roe *et al.*, *J. Am. Chem. Soc.* **106**, 1676 (1984).
15. T. E. Westre *et al.*, *J. Am. Chem. Soc.* **119**, 6297 (1997).
16. Supplemental data are available at *Science* Online at www.sciencemag.org/feature/data/1044838.shl.
17. M. T. Caudle, P. Riggs-Gelasco, A. K. Gelasco, J. E. Penner-Hahn, V. L. Pecoraro, *Inorg. Chem.* **35**, 3577 (1996).
18. The refined Fe-O distances differ only by the estimated resolution of the data ($\pi/2k_{max} \approx 0.13$ Å), which further indicates that these fits are not chemically meaningful.
19. W. Liu and H. H. Thorp, *Inorg. Chem.* **32**, 4102 (1993).
20. The Debye-Waller factor was constrained to be the same as those found for the 25-ms sample to permit quantitative comparison of the coordination numbers.
21. L. Shu *et al.*, *Science* **275**, 515 (1997).
22. Y. Dong, Y. Zang, L. Shu, E. C. Wilkinson, L. Que Jr., *J. Am. Chem. Soc.* **119**, 12683 (1997).
23. K. Kim and S. J. Lippard, *J. Am. Chem. Soc.* **118**, 4914 (1996).
24. Y. Dong, S. Yan, V. G. Young Jr., L. Que Jr., *Angew. Chem. Int. Ed. Engl.* **35**, 618 (1996).
25. T. Ookubo *et al.*, *J. Am. Chem. Soc.* **118**, 701 (1996).
26. E. Larson, M. S. Lah, X. Li, J. A. Bonadies, V. L. Pecoraro, *Inorg. Chem.* **31**, 373 (1992).
27. J. Ling *et al.*, *J. Am. Chem. Soc.* **116**, 7682 (1994).
28. M. J. Baldwin *et al.*, *J. Am. Chem. Soc.* **114**, 10421 (1992).
29. U. Bossek, T. Weyhermüller, K. Wieghardt, B. Nuber, J. Weiss, *J. Am. Chem. Soc.* **112**, 6387 (1990).
30. This assumes that the Fe-O-O-Fe torsion angle is zero. γ would be even smaller for nonzero torsion angles.
31. T. C. Brunold, N. Tamura, N. Kitajima, Y. Moro-oka, E. I. Solomon, *J. Am. Chem. Soc.* **120**, 5674 (1998).
32. G. S. Waldo and E. C. Theil, *Biochemistry* **32**, 13261 (1993); X. Yang, Y. Chen-Barrett, P. Arosio, N. D. Chasteen, *Biochemistry* **37**, 9743 (1998).
33. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; F, Phe; H, His; Q, Gln; S, Ser; and W, Trp.
34. Y. Ha, D. Shi, G. W. Small, E. C. Theil, N. M. Allewell, *J. Biol. Inorg. Chem.* **4**, 243 (1999); P. Hempstead *et al.*, *J. Mol. Biol.* **268**, 424 (1997).
35. L. F. Dickey *et al.*, *J. Biol. Chem.* **262**, 7901 (1987); A. Treffry, Z. Zhao, M. A. Quail, J. R. Guest, P. M. Harrison, *Biochemistry* **34**, 15204 (1995); J. Fetter, J. Cohen, D. Danger, J. Sanders-Loehr, E. C. Theil, *J. Biol. Inorg. Chem.* **2**, 652 (1997). Kinetics of diferric peroxo formation and decay in a QXXA ferritin (human H) differ from either the QXXD (frog M) or QXXS (frog H); no information is available for the QXXQ ferritin (maize). In the *Escherichia coli* ferritins, EXXH occurs instead of QXXD, QXXA, or QXXS as the ligands to the second Fe, and the Fe is retained at the diiron site [A. Treffry, Z. Zhao, M. A. Quail, J. R. Guest, P. M. Harrison, *FEBS Lett.* **432**, 213 (1998)].
36. We thank K. Herlihy, G. W. Small, and A. R. Tipton for assistance in the preparation of the ferritin samples and B. G. Fox for communicating results before publication. Supported in part by grants from NIH (GM-45205 to J.E.P.-H., GM-47295 and GM-58778 to B.H.H. and D.E.E., DK-20251 to E.C.T.). X-ray absorption data were measured at Stanford Synchrotron Radiation Laboratory, which is operated by the U.S. Department of Energy (DOE), Office of Basic Energy Sciences, with additional support from NIH, National Center for Research Resources, and the DOE Office of Biological and Environmental Research.

24 August 1999; accepted 16 November 1999

Modulation of Brain Reward Circuitry by Leptin

Stephanie Fulton, Barbara Woodside, Peter Shizgal*

Leptin, a hormone secreted by fat cells, suppresses food intake and promotes weight loss. To assess the action of this hormone on brain reward circuitry, changes in the rewarding effect of lateral hypothalamic stimulation were measured after leptin administration. At five stimulation sites near the fornix, the effectiveness of the rewarding electrical stimulation was enhanced by chronic food restriction and attenuated by intracerebroventricular infusion of leptin. In contrast, the rewarding effect of stimulating neighboring sites was insensitive to chronic food restriction and was enhanced by leptin in three of four cases. These opposing effects of leptin may mirror complementary changes in the rewarding effects of feeding and of competing behaviors.

Research on the regulation of feeding and energy balance has been galvanized by the sequencing of the *obese* (*ob*) gene and the expression of its protein product, leptin, a circulating hormone secreted by adipocytes (*1*). Circulating leptin levels reflect the size of the fat mass (*2*), and thus, this hormone has been considered as a signal that regulates long-term energy balance. Rodents with homozygous mutations in the *ob* gene (the *ob/ob* mouse) or in the gene for the leptin receptor (the *db/db* mouse or the *fa/fa* rat) manifest profound hyperphagia and obesity. Central or peripheral administration of leptin reverses the obesity syndrome found in *ob/ob* mice, stimulates metabolism, and reduces food intake in lean mice or rats (*3*).

Among the many ways in which leptin could alter food intake is by reducing the appetitive value of food. Such changes could ensue if leptin were to alter the state of brain reward circuitry. Self-administration of rewarding electrical brain stimulation ("self-stimulation") has long been used to assess the state of this circuitry. Rats and a wide range of other vertebrates will actively seek out electrical stimulation of certain brain regions, including the lateral hypothalamus (LH) (*4*). The effect that induces the subject to reinitiate the stimulation is called "brain stimulation reward" (BSR). Weight loss resulting from chronic food restriction has been shown to enhance the rewarding effect of stimulating LH sites close to the fornix (*5*); this perifornical region has been implicated in the control of feeding and energy balance (*6*). Thus, one might expect that the rewarding effect produced by stimulation of this region would be influenced by leptin. We tested this hypothesis by measuring leptin-induced changes in self-stimulation of the perifornical hypothalamus.

In the demonstrations by Carr and his co-workers that perifornical self-stimulation is modulated by chronic food restriction (*7*), the rate at which the rats harvested the electrical rewards was measured as a function of the stimulation frequency. Chronic food restriction shifted the resulting rate-frequency function leftward, toward weaker stimulation strengths; the lower the body weight, the weaker the stimulation required to entice the rats to earn a given number of rewards. We adopted an analogous approach to determine

Center for Studies in Behavioural Neurobiology, Concordia University, Montréal, QC, H3G 1M8, Canada.

*To whom correspondence should be addressed. E-mail: shizgal@CSBN.concordia.ca

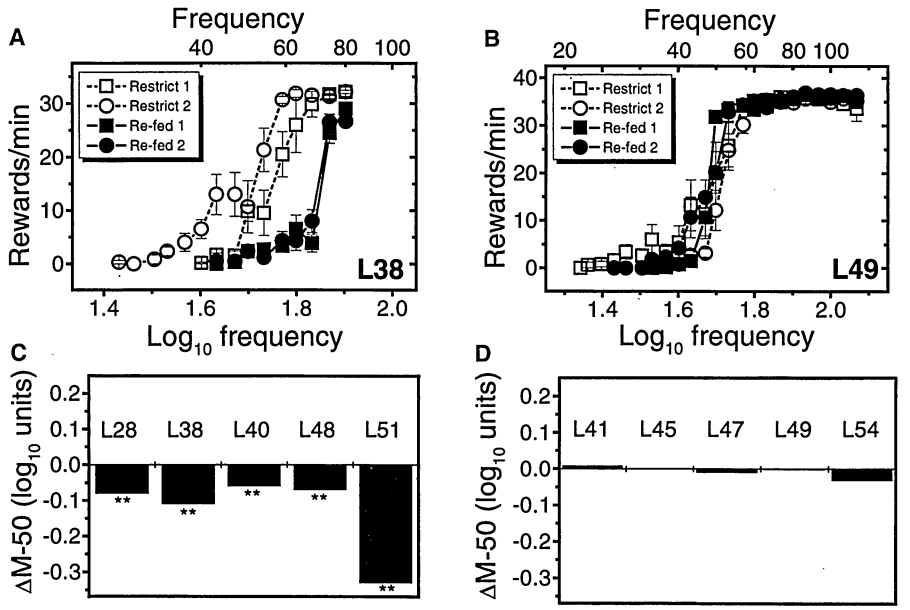

Fig. 1. Effects of chronic food restriction on self-stimulation at LH sites where the rewarding effect of electrical stimulation is sensitive (left) or insensitive (right) to chronic food restriction. (**A**) Rate-frequency curves obtained with stimulation of a perifornical site are shifted leftward during chronic food restriction (open symbols) with respect to curves obtained after subsequent refeeding (solid symbols). (**B**) In contrast, stimulation of a neighboring site yields overlapping rate-frequency curves during chronic food restriction and after refeeding. Each data point in (A) and (B) is an average of six measurements collected on each test day. Error bars indicate SEM. (**C** and **D**) Magnitude of the curve shifts produced by refeeding after chronic food restriction in all subjects. M-50 represents the stimulation frequency required to induce the rat to earn half of the maximal number of rewards available per trial. $^{**}P < 0.005$.

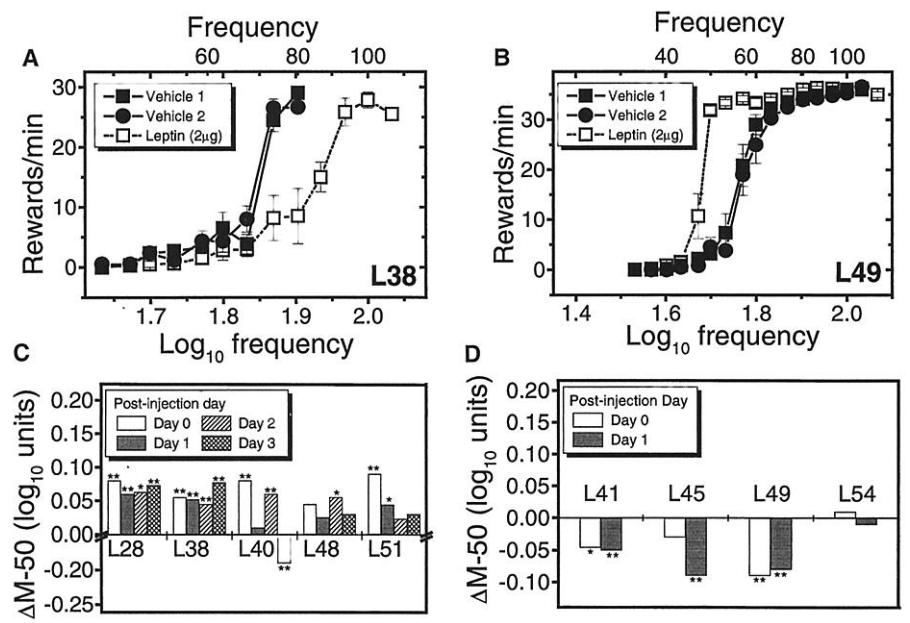

Fig. 2. Opposite influence of intracerebroventricular (ICV) infusion of leptin on rewarding effects of LH stimulation (left, sensitive to chronic restriction; right, insensitive to chronic restriction). (**A**) At a site where chronic restriction enhanced the rewarding effect (Fig. 1A), leptin shifted the rate-frequency curve rightward (leptin, open symbols; vehicle control, solid symbols). (**B**) At a site where chronic restriction failed to enhance the rewarding effect (Fig. 1B), leptin shifted the rate-frequency curve leftward. Error bars in (A) and (B) indicate SEM. (**C**) Magnitude of curve shifts (ΔM-50) during the 4 days after ICV leptin at five sites where the rewarding effect was enhanced by chronic food restriction. (There is a break in the *y* axis between 0 and -0.155 $\log_{10}$ units.) (**D**) Magnitude of curve shifts during the 2 days after ICV leptin at four sites where the rewarding effect was not altered by chronic food restriction. $^{*}P < 0.05$; $^{**}P < 0.005$.

whether leptin modulates the rewarding effect of perifornical stimulation.

Male Long-Evans rats bearing chronic stimulating electrodes and cerebroventricular cannulas (*8*) self-stimulated by pressing a lever that triggered a 1-s train of rectangular, constant-current pulses, 0.1 ms in duration. The stimulation frequency was varied across trials over a range that drove the number of rewards earned from maximal to minimal levels (*9*) (Fig. 1, A and B). The measure of the effectiveness of the rewarding stimulation was the frequency that produced a half-maximal rate of reward delivery ("M-50") (*10*). Manipulations that potentiate BSR decrease the M-50 value.

Before leptin treatment, BSR data were obtained under the influence of chronic food restriction. Daily food intake was limited to 10 g/day until body weight reached ~75% of the weight of age-matched controls. Rate-frequency curves collected during this period of restriction were compared to those obtained during a later stage of the experiment, when body weight had returned to normal levels after a period of free feeding (*11*).

In five subjects, chronic food restriction enhanced BSR. As illustrated in Fig. 1A, rate-frequency curves obtained in these rats during food restriction lie to the left of the curves obtained after subsequent refeeding, and the M-50 values (Fig. 1C) declined by 0.07 to 0.33 $\log_{10}$ units. In contrast, refeeding after food restriction had little effect in the remaining five subjects. The rate-frequency curves obtained during restriction in these rats overlap the curves obtained during subsequent free feeding (for example, Fig. 1B), and the M-50 values remained relatively stable (Fig. 1D). Taken together, the results are consistent with previous reports (*5*) that food restriction facilitates self-stimulation only at certain LH sites.

The effects of leptin on self-stimulation were examined at the end of the period of chronic food restriction, when body weight was ~75% of control values. One hour before the test sessions, 2 μg of recombinant murine leptin (Peprotech, Roanoke, Virginia) dissolved in 1.6 μl of water was infused into the right lateral cerebral ventricle over a 2-min period. In a separate group of rats, this dose produced a reliable reduction in dark-phase food intake over a period of 4 hours (*12*). In the five rats that had shown enhancement of BSR during chronic food restriction, leptin decreased the effectiveness of the rewarding stimulation (*13*). Whereas chronic food restriction produced leftward shifts in the rate-frequency curves obtained from these subjects, leptin produced rightward shifts (Fig. 2, A and C) (*14*). These leptin-induced rightward shifts persisted for as long as 4 days after a single infusion. Leptin had the opposite effect in three of four rats in which the rewarding effect of LH stimulation was

unresponsive to food restriction. In these three rats, leptin increased the effectiveness of the rewarding stimulation: The rate-frequency curves were shifted leftward (Fig. 2, B and D). In one rat in which BSR was unresponsive to chronic food restriction, the rate-frequency curves were not altered significantly by leptin administration; the effect of leptin could not be tested in the remaining restriction-insensitive subject (*15*).

After completion of testing, the LH stimulation sites were marked by means of the Prussian blue method (*16*). Sites where BSR was enhanced by chronic food restriction and diminished by leptin were located dorsal or dorsolateral to the fornix (Fig. 3). The remaining sites were nearby but nonoverlapping. Such a distribution is consistent with the notion that the rewarding effect of LH stimulation arises from activation of multiple, functionally different subpopulations of inhomogeneously intertwined neurons (*17*). In this view, small differences in the location of the electrode tip and the path of current flow could alter the relative weights of the subpopulations sampled by different electrodes. In the simplest account of the results reported here, one of the stimulated subpopulations consists of neurons that arise in, terminate in, or course through the perifornical hypothalamus; activation of these cells produces a rewarding effect that is enhanced by chronic restriction and attenuated by leptin.

The chronic character of the food-restriction regimen, which was in force long enough to produce substantial weight loss (~25%), was crucial to the enhancement of BSR. In contrast to the effects of the chronic regimen, acute food deprivation was ineffective in altering BSR, even when imposed for 48 hours (*18*). As shown in Fig. 4, most rate-frequency curves obtained during acute deprivation overlap curves obtained during free feeding, even in the subjects in which BSR was enhanced by chronic food restriction (Fig. 1, A and C). Thus, the enhancement of BSR by food restriction appears to depend on signals that contribute to the regulation of long-term energy balance.

The notion that BSR is modulated by signals related to the long-term rather than the short-term regulation of energy balance is consistent with previous findings. For example, at LH sites where BSR is enhanced by chronic food restriction, the rewarding effect is not altered during acute glucopenia induced by 2-deoxyglucose or during acute lipoprivation induced by nicotinic acid (*19*). It has also been shown that BSR is insensitive to acute accumulation of sucrose in the gut (*20*).

The reduction in the effectiveness of the rewarding stimulation persisted for as long as 4 days after a single injection of leptin. The long duration of this effect is consistent with a report of body weight changes lasting up to 6 days after a single injection of leptin (*21*).

Leptin attenuated BSR at restriction-sensitive sites but facilitated self-stimulation of three of the four sites where BSR was unresponsive to chronic food restriction. These opposite effects of leptin may reflect the comparative process believed to underlie behavioral allocation (*22*). In such views, the prevalence of a particular behavior, such as feeding, can be reduced either by decreasing the reward value it generates or by increasing the value of competing activities. If so, leptin could make complementary contributions to energy balance by reducing food reward while enhancing the value of behaviors incompatible with feeding. At restriction-sensitive sites, neurons that link long-term changes in energy balance to the rewarding effect of food may be prominent in generating BSR, whereas at the remaining sites, BSR may arise primarily from the activation of neurons subserving behaviors incompatible with the ingestion of energy-rich substances.

The results reported here tie the actions of leptin to modulation of brain reward circuitry. A rich basis for linking these effects to specific populations of cells has been provided by recent progress in describing the receptors, neurotransmitters, and interconnections of hypothalamic neurons. For example, the perifornical area and other regions of the LH receive projections from leptin-sensitive cells containing neuropeptides (such as neuropeptide Y, α-melanocyte–stimu-

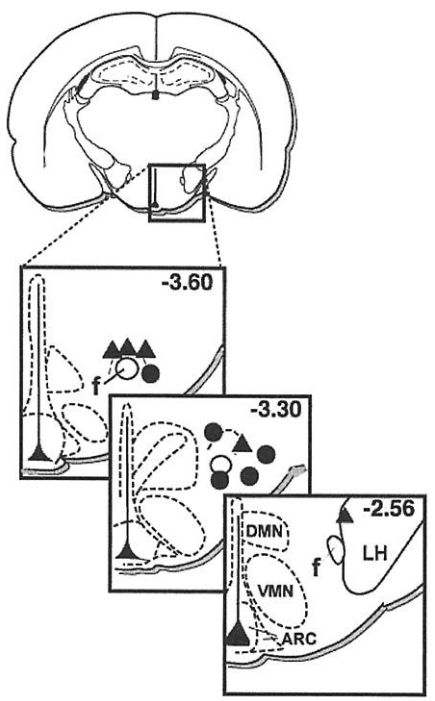

Fig. 3. Location of the tips of the stimulation electrodes. Electrodes producing rewarding effects that were enhanced by chronic food restriction are designated by solid triangles, and electrodes producing rewarding effects that were unaffected by chronic food restriction are designated by solid circles. The coronal sections are based on (*29*). f, fornix; DMN, dorsomedial nucleus of the hypothalamus; VMN, ventromedial nucleus of the hypothalamus; and ARC, arcuate nucleus.

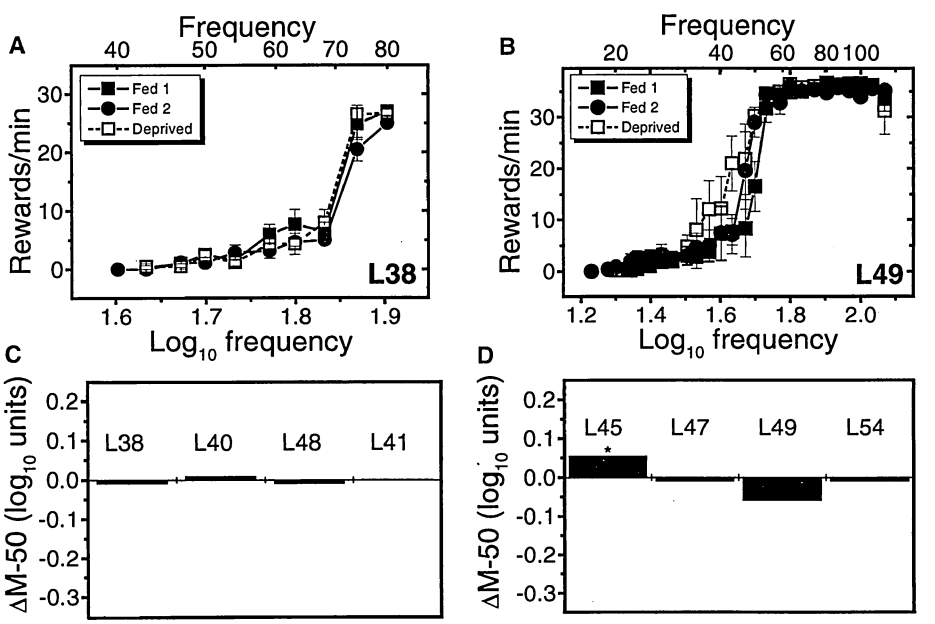

Fig. 4. Failure of acute food deprivation to alter self-stimulation. Data from sites where the rewarding effect of electrical stimulation was enhanced (left) or unchanged (right) by chronic restriction. (**A** and **B**) Neither in the case of a site where BSR was enhanced by chronic restriction (Fig. 1A) nor in the case of a site where BSR was insensitive to chronic restriction (Fig. 1B) did rate-frequency curves obtained after 48 hours of food deprivation differ systematically from the free-feeding baseline. Error bars indicate SEM. (**C** and **D**) Magnitude of the curve shifts (ΔM-50) produced by acute food deprivation (four restriction-sensitive and four restriction-insensitive sites, respectively). In the one case in which a significant effect was observed (L45), deprivation produced a small rightward shift, suggesting that the rewarding effect was attenuated. *$P < 0.05$.

lating hormone, agouti-related protein, and cocaine-amphetamine–regulated transcript) that are implicated in the control of feeding and energy balance (*23, 24*). The perifornical LH includes neurons that express the long form of the leptin receptor (*25*). Orexin or melanin-concentrating hormone (*23, 26*), neuropeptides that promote food intake and weight gain (*27*), have been found in LH neurons, and LH neurons containing corticotropin-releasing hormone have been implicated in dehydration-induced anorexia (*28*). Working out the contribution of such cells to the rewarding effects of electrical brain stimulation and feeding could prove important to understanding energy balance. Conversely, progress in understanding the neural control of food intake and energy expenditure may shed light on the structure and function of brain reward circuitry.

References and Notes

1. Y. Zhang *et al.*, *Nature* **372**, 425 (1994).
2. M. Maffei *et al.*, *Nature Med.* **1**, 1155 (1995).
3. M. A. Pelleymounter *et al.*, *Science* **269**, 540 (1995); L. A. Campfield, F. J. Smith, Y. Guisez, R. Devos, P. Burn, *Science* **269**, 546 (1995); J. L. Halaas *et al.*, *Science* **269**, 543 (1995); R. J. Seeley *et al.*, *Horm. Metab. Res.* **28**, 664 (1996).
4. J. Olds and P. M. Milner, *J. Comp. Physiol. Psychol.* **47**, 419 (1954); M. E. Olds and J. Olds, *J. Comp. Neurol.* **120**, 259 (1963).
5. J. E. Blundell and L. J. Herberg, *Nature* **219**, 627 (1969); K. D. Carr and T. Wolinsky, *Brain Res.* **607**, 141 (1993).
6. B. Stanley, W. Magdalin, A. Seirafi, W. Thomas, S. Leibowitz, *Brain Res.* **604**, 304 (1993); E. Gillard *et al.*, *J. Neurosci.* **18**, 2646 (1998); S. Leibowitz and C. Rossakis, *Brain Res.* **172**, 115 (1979); S. Leibowitz, *Brain Res.* **98**, 529 (1975).
7. G. Abrahamsen, Y. Berman, K. D. Carr, *Brain Res.* **695**, 186 (1995).
8. With skull landmarks bregma and lambda positioned on the same horizontal plane, bilateral monopolar electrodes were aimed at the perifornical LH (3 mm posterior to bregma, 1.6 mm lateral to the midsagittal sinus, and 7.8 mm below the dura mater), and a 24-gauge stainless-steel guide cannula (Plastics One, Roanoke, VA) was aimed at the right lateral ventricle (0.4 mm posterior to bregma, 1.6 mm lateral to the midsagittal sinus, and 4 mm below the dura mater). After recovery from surgery and before leptin administration, cannula placement was verified by injecting 2 μg of angiotensin II and determining whether vigorous drinking began within 5 min. For further details concerning electrodes and surgery, see A. Arvanitogiannis, L. Riscaldino, P. Shizgal, *Physiol. Behav.* **65**, 805 (1999).
9. The current was held constant throughout testing and ranged from 200 to 450 μA across subjects. The stimulation frequency was decreased from trial to trial by 0.033 $\log_{10}$ units. All testing was conducted toward the end of the dark phase of the light/dark cycle.
10. Broken-line functions, with a horizontal lower asymptote, a rising linear segment, and a horizontal upper asymptote [C. R. Gallistel and G. Freyd, *Pharmacol. Biochem. Behav.* **26**, 731 (1987)], were fit to each of the six rate-frequency curves collected daily. The frequency required to maintain a half-maximal rate of reward delivery (M-50) was derived from each of the broken-line functions. An M-50 value was retained for further analysis when the broken-line function accounted for at least 75% of the variance of the rate-frequency data. Use of this goodness-of-fit criterion eliminated <3% of the data.
11. By means of within-subject, one-way analyses of variance, a set of 12 M-50 values from the food-restriction condition was compared to 12 values obtained after return to normal body weight, as determined by comparison to the body weights of age-matched control subjects. The leptin test intervened between the gathering of the M-50 values during food restriction and the onset of the period of refeeding.
12. S. Fulton, B. Woodside, P. Shizgal, unpublished data.
13. A Dunnett test for multiple comparisons was employed to compare the 12 M-50 values from two vehicle treatment days to the six values obtained on each day after leptin administration.
14. In a subsequent test of the restriction-sensitive placements under free-feeding conditions, leptin again produced significant increases ($P < 0.005$) in M-50 values in rats L38 and L51. Smaller increases seen on 2 of 4 days in rat L28 fell short of the statistical criterion, and little change was seen in the remaining two rats (data available at www.sciencemag.org/feature/data/1044048.shl). It is not surprising that, in several rats, the effect of a fixed amount of exogenous leptin was stronger during restriction than after refeeding. Body weight overshot prerestriction levels during refeeding, and hence, circulating levels of leptin were probably much higher.
15. The cannula in rat L47 became clogged before leptin testing.
16. With the stimulating electrode serving as the anode, a 100-μA current was applied for 15 s. Rats were then injected intraperitoneally with a lethal dose of sodium pentobarbitol (100 mg/kg) and perfused intracardially with phosphate-buffered saline followed by a mixture of 10% formalin (100 ml), trychloroacetic acid (0.5 g), potassium ferrocyanide (3 g), and potassium ferricyanide (3 g). Brains were removed and stored in 10% formalin. After a 24-hour immersion in a 20% sucrose-formalin solution, the brains were frozen, sliced on a cryostat into 30-μm coronal sections, and mounted on gelatin-coated slides (Fisher Scientific). Sections were stained for Nissl substance, using formal thionin. The location of stimulation sites was identified with the aid of a stereotaxic atlas (*29*).
17. A. Arvanitogiannis, M. Waraczynski, P. Shizgal, *Physiol. Behav.* **59**, 795 (1996).
18. Acute-deprivation data for rat L28 did not meet the stability criterion, and rat L51 died before acute-deprivation testing.
19. S. Cabeza de Vaca, S. Holiman, K. D. Carr, *Physiol. Behav.* **64**, 251 (1998).
20. K. L. Conover and P. Shizgal, *Behav. Neurosci.* **108**, 559 (1994).
21. I. Cusin, F. Rohner-Jeanrenaud, A. Stricker-Kongrad, B. Jeanrenaud, *Diabetes* **45**, 1446 (1996).
22. R. Herrnstein, *J. Exp. Anal. Behav.* **13**, 243 (1970); P. Shizgal, *Curr. Opin. Neurobiol.* **7**, 198 (1997).
23. C. Elias *et al.*, *J. Comp. Neurol.* **402**, 442 (1998).
24. T. Horvath, S. Diano, A. N. van den Pol, *J. Neurosci.* **19**, 1072 (1999).
25. J. G. Mercer *et al.*, *FEBS Lett.* **387**, 113 (1996); J. K. Elmquist, C. Bjorbaek, R. S. Ahima, J. S. Flier, C. B. Saper, *J. Comp. Neurol.* **395**, 535 (1998);
26. C. Broberger, L. de Lecea, J. G. Sutcliffe, T. J. Hokfelt, *J. Comp. Neurol.* **402**, 460 (1998).
27. D. Qu *et al.*, *Nature* **380**, 243 (1996); T. Sakurai *et al.*, *Cell* **92**, 573 (1998).
28. A. G. Watts, G. Sanchez-Watts, A. B. Kelly, *J. Neurosci.* **19**, 6111 (1999).
29. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates* (Academic Press, New York, ed. 4, 1998), figures 30, 33, and 34.

30. The authors thank D. Richard for his insightful comments on an earlier draft and the "Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec" (grant 97ER0124) and the Natural Sciences and Engineering Research Council of Canada (grant OGP0000308) for research support.

29 July 1999; accepted 16 November 1999

Sex Determination in Malaria Parasites

Richard E. L. Paul,[1]* Timothy N. Coulson,[2] Anna Raibaud,[1] Paul T. Brey[1]

A century ago, W. G. MacCallum identified distinct male and female forms in malaria parasites of both birds and humans. Since then, scientists have been puzzled by the high female-to-male ratios of parasites in *Plasmodium* infections and by the mechanism of sex determination. The sex ratio of malaria parasites was shown to become progressively more male as conditions that allow motility and subsequent fertilization by the male parasites become adverse. This resulted from an increased immune response against male gametes, which coincides with intense host erythropoietic activity. Natural and artificial induction of erythropoiesis in vertebrate hosts provoked a shift toward male parasite production. This change in parasite sex ratio led to reduced reproductive success in the parasite, which suggests that sex determination is adaptive and is regulated by the hematologic state of the host.

Malaria parasites are transmitted from the vertebrate host to the mosquito vector by sexual blood stages (gametocytes). When taken up in the bloodmeal by the engorging female mosquito, male gametocytes (microgametocytes) undergo exflagellation, producing up to eight male gametes; a female gametocyte (macrogametocyte) produces only one female gamete, which is fertilized by a single male gamete. The gametocyte sex ratio tends to be female-biased in all species of malaria parasites (*1*) and several authors have considered that the theory of local mate competition, which successfully explains many other cases of biased sex ratios (*2*), determines the gametocyte sex ratio of malaria parasites (*3*). According to this theory, when an infection consists of a few parasite clones, whose offspring will mate among themselves, a

[1]Laboratoire de Biochimie et Biologie Moléculaire des Insectes, Institut Pasteur, 25 Rue du Dr. Roux, 75724 Paris Cedex 15, France. [2]Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, United Kingdom.

*To whom correspondence should be addressed. E-mail: topotito@pasteur.fr