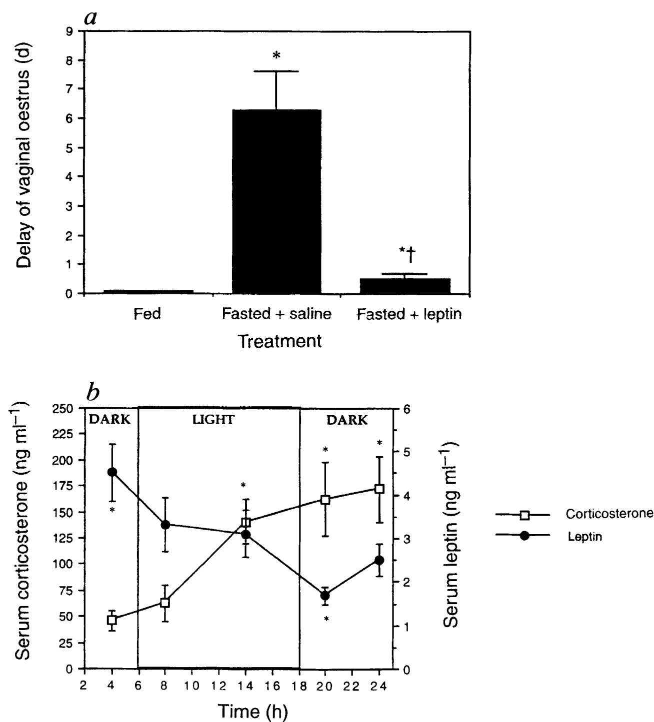

FIG. 2 *a*, Effect of fasting and leptin treatment on the oestrus cycle. Female C57BJ mice were housed singly in plastic cages under ambient conditions, with a 12 h light (06:00–1800) and 12 h dark (18:00–06:00) cycle, and free access to chow and water. Daily vaginal smears were obtained from age 8 weeks for the duration of 3 oestrus cycles. Thirty females with regular 4–5 day oestrus cycles were assigned to 3 treatment groups (10 per group). One group was fed *ad libitum*, and the others were fasted for 48 h during dioestrus, and treated with twice daily i.p. injections of recombinant mouse leptin, 1 μg per g body weight or saline. Body weight decreased by 15%, from 23.0 ± 0.9 to 19.6 ± 0.9 gm, and leptin treatment did not alter this. Twelve h after the last injection, all mice were allowed free access to food. Body weight was regained to 98% of control after 24 hours and fully restored after 48 hours. Daily vaginal smears were obtained, each female serving as its own control. The delay of vaginal oestrus was determined as the difference between the length of the cycle (oestrus to oestrus) before and after treatment[11]. Data are means ± s.e.m., $n = 10$ per group. *$P < 0.05$ compared with fed controls; †$P < 0.05$ compared with fasted mice by ANOVA and Fisher PSLD. *b*, Diurnal variation of serum leptin and corticosterone. Male C57BL mice were used (Table 1), and handling was restricted to cage cleaning. Seventy per cent of food intake (2.78 ± 0.18 g chow per mouse) occurred during the dark cycle, and 30% (1.19 ± 0.10 g chow per mouse) during the light cycle. Groups of mice ($n = 5$) were killed by decapitation at 04:00, 08:00, 14:00, 20:00 and 24:00 h. Serum corticosterone and leptin were measured by radioimmunoassay (ICN and Linco, respectively). Data are means ± s.e.m., *$P < 0.05$ compared with 08:00 h by ANOVA and Fisher PSLD.

were similar to those of fed controls, recombinant leptin may be less potent, or the leptin radioimmunoassay may overestimate bioactive leptin. Alternatively, as falling insulin may mediate adaptation to starvation through regulation of hypothalamic NPY[9], leptin and insulin may cooperate to regulate aspects of the neuroendocrine response to starvation.

In an environment where periodic limitations of food availability, rather than continuous access, is common, the ability to adapt to starvation is fundamentally important to survival of the species. These studies show that falling leptin concentration is a critical signal that initiates the neuroendocrine response to starvation, including limiting procreation, decreasing thyroid thermogenesis, and increasing secretion of stress steroids, which together are likely to have survival value during prolonged nutritional deprivation. Given the high prevalence of apparent leptin resistance in obese rodents[5,6] and humans[6,7], the physiological response to decreasing leptin concentration with starvation may be the dominant role of this hormone. □

Received 29 April; accepted 30 May 1996.

1. Zhang, Y. *et al. Nature* **372,** 425–432 (1994).
2. Halaas, J. L. *et al. Science* **269,** 543–546 (1995).
3. Campfield, L. A. *et al. Science* **269,** 546–549 (1995).
4. Stephens, T. W. *et al. Nature* **377,** 530–532 (1995).
5. Frederich, R. C. *et al. Nature Med.* **1,** 1311–1314 (1995).
6. Maffei, M. *et al. Nature Med.* **1,** 1155–1161 (1995).
7. Considine, R. V. *et al. N. Engl. J. Med.* **334,** 292–295 (1996).
8. Cahill, J. G. *et al. J. clin. Invest.* **45,** 1751–1769 (1966).
9. Schwartz, M. W., Dallman, M. F. & Woods, S. C. *Am. J. Physiol.* **269,** R949–R957 (1995).
10. Dallman, M. F. *et al. Front. Neuroendocr.* **14,** 303–347 (1993).
11. Bronson, F. H. & Marstellar, F. *Biol. Reprod.* **33,** 660–667 (1985).
12. Connors, J. M. *et al. Endocrinology* **117,** 900–906 (1985).
13. Frederich, R. C. *et al. J. clin. Invest.* **96,** 1658–1663 (1995).
14. Bray, G. A., Fisler, J. & York, D. A. *Front. Neuroendocr.* **14,** 128–181 (1990).
15. Schreihofer, D. A., Amico, J. A. & Cameron, J. L. *Endocrinology* **132,** 1890–1897 (1993).
16. Bates, G. M. *Clin. Obstet. Gynec.* **28,** 632–644 (1985).
17. Tartaglia, L. A. *et al. Cell* **83,** 1263–1271 (1995).
18. Lee, G.-H. *et al. Nature* **379,** 632–635 (1996).
19. White, J. D. & Kershaw, M. *Molec. cell. Neurosci.* **1,** 41–48 (1989).
20. Pierroz, D. Q. *et al. Endocrinology* **137,** 3–12 (1996).
21. Chebab, F. F., Lim, M. E. & Lu, R. *Nature Genet.* **12,** 318–320 (1996).
22. Erickson, J. C., Clegg, K. E. & Palmiter, R. D. *Nature* **381,** 415–418 (1996).
23. Cooper, R. L. *et al. Endocrinology* **114,** 391–396 (1984).
24. Qu, D. *et al. Nature* **380,** 243–247 (1996).

ACKNOWLEDGEMENTS. We thank J. Hurwitz for technical assistance and Eli Lilly and Co. for supplying the recombinant leptin. This work was supported by a grant from the NIH to J.S.F. and ADA to E.M.F.

CORRESPONDENCE and requests for materials should be addressed to J.S.F. (e-mail: jflier@bih.harvard.edu).

Consolidation in human motor memory

Thomas Brashers-Krug*‡, Reza Shadmehr† & Emilio Bizzi*

* Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
† Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland 21205, USA

LEARNING a motor skill sets in motion neural processes that continue to evolve after practice has ended, a phenomenon known as consolidation[1–4]. Here we present psychophysical evidence for this, and show that consolidation of a motor skill was disrupted when a second motor task was learned immediately after the first. There was no disruption if four hours elapsed between learning the two motor skills, with consolidation occuring gradually over this period. Previous studies in humans and other primates have found this time-dependent disruption of consolidation only in explicit memory tasks[5–12], which rely on brain structures in the medial temporal lobe[9,13,14]. Our results indicate that motor memories, which do not depend on the medial temporal lobe[8,15], can be transformed by a similar process of consolidation. By extending the phenomenon of consolidation to motor memory, our results indicate that distinct neural systems share similar characteristics when encoding and storing new information.

Subjects moved the handle of a two-link planar manipulandum[16] (Fig. 1*a*) to guide a cursor to a series of 192 targets (one target set) that appeared one at a time on a computer monitor mounted above the manipulandum (Fig. 1*b*). On the first day of testing (day 1), after baseline trajectories were recorded (Fig. 1*b*),

‡ Present address: Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland 21287, USA.

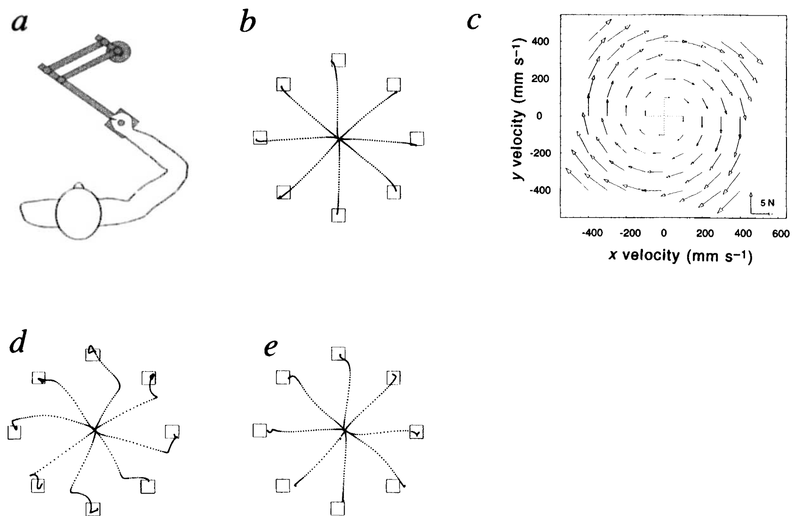

FIG. 1 We trained 70 naive, right-handed subjects between the ages of 18 and 35 years in a motor learning task[16] (5 subjects were excluded from final analysis because they failed to follow the instructions). Subjects learned to make reaching movements while interacting with a force-producing manipulandum. *a*, Overhead view of the experimental setup. The apparatus is a two-joint planar manipulandum powered by two torque motors mounted at its base[30]. *b*, Typical hand trajectories for one subject. Subjects moved the handle of the manipulandum 10 cm to targets that appeared in one of eight directions: four directions starting from the centre of the monitor (0°, 45°, 90°, 135°) and the four corresponding directions back to the centre from each of those targets (180°, 225°, 270°, 315°). Subjects were instructed to bring the cursor (representing the position of their hand, displayed on a monitor facing the subjects) in a straight line to each target with a movement time of 500 ± 50 ms. The computer generated a distinctive sound if a subject reached the target within the allotted time. A target turned blue if a subject reached it too slowly, red if too quickly. After 5–10 min practice, when subjects could move at the required pace, we recorded the position and velocity of the manipulandum at 100 Hz for each subject while the subject made 12 movements (baseline trajectories) in each of the 8 target directions. There were no perturbing forces during these movements. *c*, Clockwise velocity-dependent forces imposed by the manipulandum plotted as a function of hand velocity. The direction and length of an arrow indicate the direction and magnitude of the forces, respectively, in each location in velocity space. The movements of half of the subjects were perturbed by clockwise forces, half by anticlockwise forces. Forces were calculated linearly as a function of hand velocity: $f = \mathbf{B}\dot{x}$. The matrix $\mathbf{B}$ was either $\begin{bmatrix} 0 & 13 \\ -13 & 0 \end{bmatrix}$ N s m^{-1} or $\begin{bmatrix} 0 & -13 \\ 13 & 0 \end{bmatrix}$ N s m^{-1}. *d*, Trajectories of one subject when the clockwise perturbing forces were first turned on. *e*, Trajectories of the same subject after 5 min practice in the presence of the clockwise velocity-dependent forces.

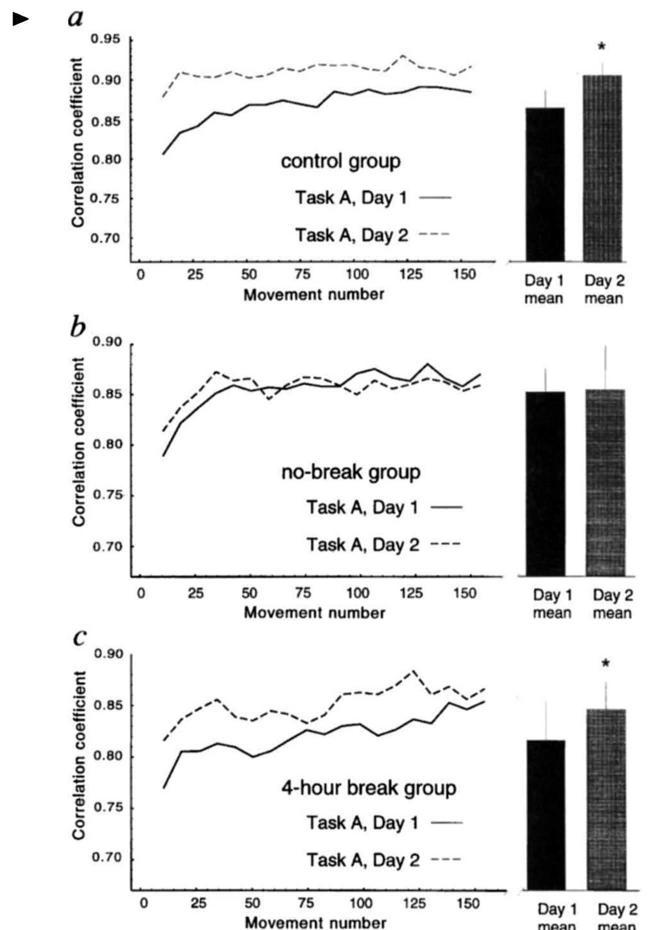

FIG. 2 Learning curves for three groups of subjects. The similarity between two trajectories was quantified using a correlation measure that treated each trajectory as a sequence of velocity vectors ($\dot{x}$ sampled at 10-ms intervals)[16]. The value of the correlation coefficient, which depends on both the path and speed of a movement, can vary between −1 and +1, with +1 indicating that two movements are completely identical. Performance, however, was typically bounded by values of the correlation coefficient between 0.65 and 0.93. Learning curves in task A on days 1 and 2 are shown: *a*, for the control group; *b*, for the no-pause group (task B performed immediately after completion of task A); and *c*, for the 4-hour break group (task B performed 4 hours after completion of task A). The mean performances on days 1 and 2 are shown in bars on the right of each graph (same scale as for learning curves). Mean performance on day 2 was significantly higher than on day 1 for the control and 4-hour break groups, but not for the no-break group. There was no significant difference between the performance of task A on day 1 between these groups. Because subjects were not told in advance of a target set which forces would be applied, the first movement in each target direction was treated as a cue to the pattern of the forces, and was not included in the analysis. (Inclusion of these data, however, did not alter the significance of the comparisons). The asterisk indicates that mean performance on day 2 was significantly higher than on day 1; statistical significance determined with two-tailed *t*-test, with Bonferroni correction for multiple-planned comparisons.

the manipulandum perturbed subjects' movements with a pattern of velocity-dependent forces during one target set (task A) (Fig. 1*c*, *d*). The motor learning task required subjects to compensate for these imposed forces[16]. By the end of the target set, subjects were able to guide the cursor accurately to the targets despite the perturbing forces (Fig. 1*e*). The similarity between a subject's movements in the presence of the forces and his or her baseline movements indicated how well that subject learned to compensate for the forces (Fig. 2).

After the target set, subjects were divided into 6 groups, with 12 subjects in 5 of the groups and 10 in the other. We tested the first

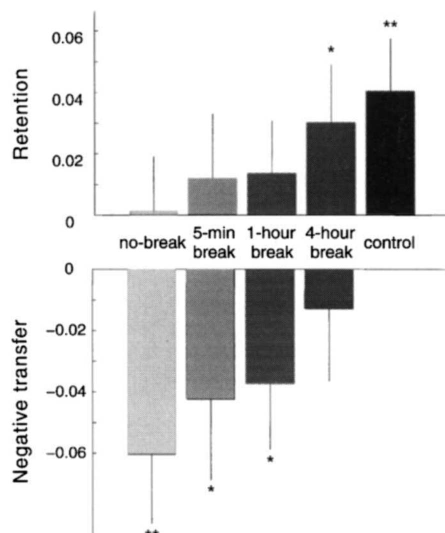

FIG. 3 Retention of the task A motor skill and transfer to task B as a function of temporal distance between tasks A and B. Controls did not perform task B. Retention was calculated as the difference between the mean performance in task A on days 2 and 1. Transfer was calculated as the difference between mean performance in task B and task A, both on day 1. An analysis of variance (ANOVA) performed on the retention data indicated a significant difference between the means: $F(4,48) = 3.05$, $P < 0.05$. An ANOVA performed on the negative transfer data also indicated a significant difference between the means: $F(3,37) = 2.88$, $P < 0.05$. The asterisk indicates significant difference from 0, $P < 0.05$; two asterisks significant difference from 0, $P < 0.005$; three asterisks indicates significant difference from 0, $P < 0.001$; corrected for multiple comparisons with Bonferroni correction. The concomitant decline of negative transfer and retrograde interference seen in the group data suggests a relation between the two phenomena. This is borne out by the significant correlation ($r = 0.55$, $P < 0.0005$) between negative transfer and retrograde interference in each individual subject.

group of subjects (control group) 24 hours later (day 2) with the same forces they had learned in task A. This control group showed both retention of the motor skill and additional learning, performing at a significantly higher level on day 2 than they had on day 1 (Fig. 2).

The second group of subjects was trained on day 1 with a different pattern of forces (task B) immediately after task A (no-break group). In task B, the manipulandum produced forces opposite in direction to those applied during task A. The mean performance of these subjects in task B was significantly less than it had been in task A, an effect known as negative transfer[17]. The no-break group was also tested for retention of task A 24 hours later on day 2. In contrast to the control subjects, their mean performance was not significantly better than it had been on the previous day (Fig. 2). This occurred despite the fact that the task provided subjects with continuous visual and kinesthetic feedback on the pattern of forces present. Thus subjects were unable to benefit from their previous training, suggesting that learning task B disrupted the retention of the motor skill that had been learned in task A, a phenomenon known as retrograde interference[17].

To test whether retrograde interference could be caused by any motor task performed immediately after task A, the third group of subjects was tested in a protocol similar to that of the no-break group, the only difference being that, when subjects guided the cursor to the targets in task B, the manipulandum produced no perturbing forces. When tested for retention of task A the following day, these subjects did not demonstrate retrograde interference, performing at a significantly higher level on day 2 than they had on day 1 ($P < 0.05$; data not shown).

We next investigated whether the susceptibility of motor learning to negative transfer and retrograde interference decreased with time. We trained the final three groups of subjects in task B either 5 minutes (5-minute group), one hour (1-hour group), or four hours (4-hour group) after task A on day 1. Both retrograde interference and negative transfer decreased monotonically as the interval between tasks A and B increased (Fig. 3). Although the 5-minute and 1-hour groups performed better in task A on day 2 than they had on day 1, their retention did not approach statistical significance. Both of these groups demonstrated significant negative transfer in task B. When 4 hours passed before task B was learned, however, retention of task A was significant, and there was no significant negative transfer (Figs 2 and 3). Further, there was no difference in the amount of retention between the 4-hour group and the control group ($P > 0.05$); that is, after 4 hours had passed, skill in task A was not disrupted when task B was learned. The initial learning had consolidated.

An alternative explanation for the disappearance of retrograde interference with the passage of time would propose that subjects tended to consider two tasks learned in close succession as a single task: the confusion between the two tasks would diminish as the tasks were separated in time. This explanation places the relevant processes in the cognitive domain, rather than positing a time-dependent change in the way motor memory is stored. Further experiments, however, argued against this interpretation; practising task B 2–3 minutes after task A did not cause retrograde interference (that is, there was still retention of task A) if the subjects had already learned task A on the previous day, and thus had had 24 hours to consolidate their learning (data not shown).

Although several studies have reported the disruption of motor memories[18,19], we believe this to be the first evidence that human motor memory, one type of implicit memory, is rapidly transformed with the passage of time, and, in the absence of further practice, from an initial fragile state to a more solid state. Two broad mechanisms may account for this consolidation: either the same synapses that are altered during learning a motor skill are further altered during the changes that lead to the consolidation of that skill[1]; or new synapses are recruited to store the skill in its long-term state[20–22]. Studies using localized cerebral lesions have suggested that consolidation can occur as a result of a change in the anatomical locus of a memory trace or of changes that rely on the integrity of the medial temporal lobe[10,23]. These mechanisms, however, seem to take place over a much longer time scale (days to weeks to years) than the 4 hours we observed in our study. Alternatively, the synapses that are altered during learning of a motor skill may be further altered during the changes that lead to the consolidation of that skill[1,24]. The time course of this latter type of consolidation is consistent with the time course of our results[1,3,24–29]. □

Received 15 December 1995; accepted 13 May 1996.

1. Bailey, C. H. & Kandel, E.R. in *The Cognitive Neurosciences* (ed. Gazzaniga, M.) 19–36 (MIT Press, Cambridge, MA, 1995).
2. Karni, A. & Sagi, D. *Nature* **365,** 250–252 (1993).
3. Tully, T., Preat, T., Boynton, S. C. & Del Vecchio, M. *Cell* **79,** 35 (1994).
4. Weingartner, H. & Parker, E. (eds) *Memory Consolidation: Psychobiology of Cognition* (L. Erlbaum, Hillsdale, 1984).
5. Muller, G. & Pilzecker, A. *Z. F. Psychol.* **1,** 1–228 (1900).
6. Skaggs, E. *Psychol. Monogr.* **34,** (1925).
7. Sisson, D. E. *J. exp. Psychol.* **25,** 228–233 (1939).
8. Milner, B. *Neuropsychologia* **3,** 317–338 (1965).
9. Squire, L. R. & Alvarez, P. *Curr. Opin. Neurobiol.* **5,** 169–177 (1995).
10. Zola-Morgan, S. & Squire, L. R. *Science* **250,** 288–290 (1990).
11. McGeoch, J. & Irion, A. *Am. J. Psychol.* **43,** 579–592 (1931).
12. Squire, L. R., Slater, P. & Chace, P. *Science* **187,** 77–79 (1975).
13. Malamut, B. L. Saunders, R. C. & Mishkin, M. *Behavl Neurosci.* **98,** 759–769 (1984).
14. Zola-Morgan, S. & Squire, L. R. *Behavl Neurosci.* **99,** 22–34 (1985).
15. Corkin, S. *Neuropsychologia* **6,** 255–265 (1969).
16. Shadmehr, R. & Mussa-Ivaldi, F. A. *J. Neurosci.* **14,** 3208–3224 (1994).

17. McGeoch, J. & Irion, A. *Psychology of Human Learning* 2nd edn, 299–467 (Longmans Breen, New York, 1952).
18. Lewis, D., Smith, N. & McAllister, D. *J. exp. Psychol.* **44,** 247–260 (1952).
19. Shea, J. & Titzer, R. *J. Mot. Behav.* **25,** 264–274 (1993).
20. Alvarez, P. & Squire, L. R. *Proc. natn. Acad. Sci. U.S.A.* **91,** 7041–7045 (1994).
21. McClelland, J. L., McNaughton, B. L. & O'Reilly, R. C. *Psychol. Rev.* **102,** 419–457 (1995).
22. Mishkin, M., Malamut, B. & Bachevalier, J. *Neurobiology of Learning and Memory* (eds Lynch, G., McGaugh, J. L. & Weinberger, N. M.) 65–77 (Guilford, London, 1984).
23. Kim, J. J., Clark, R. E. & Thompson, R. F. *Behavl Neurosci.* **109,** 195–203 (1995).
24. Fujii, S., Saito, K., Miyakawa, H., Ito, K. & Kato, H. *Brain Res.* **555,** 112–122 (1991).
25. Bailey, C. H. & Chen, M. *Science* **220,** 91–93 (1983).
26. Castro-Alamancos, M. A., Donoghue, J. P. & Connors, B. W. *J. Neurosci.* **15,** 5324–5333 (1995).
27. Kimura, A., Caria, M. A., Melis, F. & Asanuma, H. *Neuroreport* **5,** 2372–2376 (1994).
28. Ito, M. *Can. J. neurol. Sci.* **20,** Suppl. 3, 70–74 (1993).
29. Seeds, N. W., Williams, B. L. & Bickford, P. C. *Science* **270,** 1992–1994 (1995).
30. Faye, I. thesis, MIT (1986).

ACKNOWLEDGEMENTS. We thank E. Fasse, T. Hodgson and N. Hogan for technical support using the MIT manipulandum; M. Smith, K. Thoroughman and K. Akhavan-Toyserkani for technical support using the Johns Hopkins manipulandum; F. Mussa-Ivaldi, S. Corkin and A. Gentile for discussions; and J. Locascio for statistical assistance. This work was supported by grants from the NIH and the ONR to E.B., and from the Whitaker Foundation and the ONR to R.S.

CORRESPONDENCE and requests for materials should be addressed to E.B. (e-mail: emilo@ai.mit.edu).

Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs

Francesco E. Pontieri*, Gianluigi Tanda†, Francesco Orzi*‡ & Gaetano Di Chiara†

* Department of Neuroscience, University 'La Sapienza', Viale dell'Université 30, 00185 Rome, Italy
† Department of Toxicology and CNR Centre for Neuropharmacology, University of Cagliari, Viale A. Diaz 182, 09126 Cagliari, Italy
‡ INM 'Neuromed', Pozzilli (IS), Italy

The question of whether nicotine, the neuroactive compound of tobacco, is addictive has been open to considerable scientific and public discussion. Although it can serve as a positive reinforcer in several animal species, including man, nicotine is thought to be a weak reinforcer in comparison with addictive drugs such as cocaine and heroin[1,2], and has been argued to be habit forming but not addictive[3,4]. Here we report that intravenous nicotine in the rat, at doses known to maintain self-administration, stimulates local energy metabolism, as measured by 2-deoxyglucose autoradiography, and dopamine transmission, as estimated by brain microdialysis, in the shell of the nucleus accumbens. These neurochemical and metabolic effects are qualitatively similar to those of other drugs, such as cocaine, amphetamine and morphine, which have strong addictive properties[5-7]. Our results provide functional and neurochemical evidence that there are specific neurobiological commonalities between nicotine and addictive drugs.

Dopamine neurotransmission in the mesolimbic system, and particularly in the nucleus accumbens, is currently recognized as a critical target of drugs of abuse[8-10]. Indeed most if not all drugs abused by humans stimulate dopamine transmission in the nucleus accumbens[11], a property that has been related to their addictive properties[8-10].

The nucleus accumbens is subdivided into a ventromedial 'shell' and a dorsolateral 'core'[12,13]. The shell is thought to be involved in the integration and expression of emotions, through its projections to the extended amygdala, lateral hypothalamus and central grey matter, and the core is thought to be involved in somatomotor functions[12,13].

Several addictive substances such as cocaine, amphetamine and morphine have previously been administered intravenously to freely moving rats at doses that sustain self-administration, and have been shown to increase preferentially or selectively the levels of extracellular dopamine[7] and energy metabolism[5,6] in the shell of the nucleus accumbens. Enhanced energy metabolism and increased dopamine transmission in the shell may therefore represent distinctive neurobiological markers of the addictive potential of drugs independently from their specific mechanism of action.

We decided to investigate whether nicotine produces neurochemical effects in the shell that resemble those of typically addictive drugs. Therefore, we studied the effects of nicotine in

TABLE 1 Effects of nicotine on cerebral glucose utilization

Brain area	Nicotine dose (mg per kg) 0 (saline control) ($n = 4$)	0.025 ($n = 4$)	0.050 ($n = 4$)
Nucleus accumbens shell	80 ± 4	92 ± 5	98 ± 4*
Nucleus accumbens core	85 ± 4	83 ± 3	87 ± 5
Ventral tegmental area	61 ± 2	62 ± 3	65 ± 3
Medial prefrontal cortex	75 ± 7	75 ± 4	71 ± 4
Caudate–putamen (dorsolateral)	109 ± 11	102 ± 6	109 ± 7
Caudate–putamen (dorsomedial)	110 ± 14	102 ± 6	111 ± 7
Caudate–putamen (ventral)	93 ± 8	90 ± 7	89 ± 4
Globus pallidus (dorsal)	60 ± 6	55 ± 7	54 ± 4
Globus pallidus (ventral)	53 ± 5	53 ± 5	52 ± 4
Central amygdala	52 ± 4	46 ± 3	47 ± 3
Basolateral amygdala	88 ± 7	88 ± 6	92 ± 10
Superior colliculus (external)	82 ± 5	94 ± 6	101 ± 10
Lateral geniculate body	89 ± 8	91 ± 5	100 ± 16

Data represent means ±s.e.m. (number of rats in parentheses). Asterisks indicate significant variations with respect to values measured in the control group ($P < 0.05$, one-way analysis of variance, followed by Tukey's t-test for multiple comparison). Nicotine, which was administered intravenously to freely moving rats, failed to modify cerebral energy metabolism in the remaining structures: anterior cingulate cortex, sensorimotor cortex, auditory cortex, visual cortex, hippocampus, cerebellar cortex, septal nuclei, lateral hypothalamus, subthalamic nucleus, substantia nigra, thalamic nuclei, habenula, medial geniculate body, inferior colliculus, pontine grey, and corpus calosum.