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25. CHO extracts were prepared from ~3 ml of centrifuged cells with 40 ml of buffer A containing leupeptin and antipain each at 20 μg/ml. The extracts were rotated for 30 min at 4°C. Insoluble material was removed by centrifugation for 45 min at 180,000*g* and 4°C, and the resulting supernatants were frozen in liquid nitrogen. Thawed CHO extract was recentrifuged and 800 μl in buffer A were incubated for 3 hours at 4°C (with rotation) with the beads containing bound GST fusion proteins. The beads were washed three times with 1 ml of buffer A and once with 50 mM Hepes (pH 7.2). After aspiration with a Hamilton syringe, bound proteins were eluted by boiling in reducing SDS sample buffer. SDS–polyacrylamide gel electrophoresis and immunoblot analysis by enhanced chemiluminescence (ECL, Amersham) were performed as described [S. Hara-Kuge *et al.*, *J. Cell Biol.* **124**, 883 (1994)]. Antibodies were used at the following dilutions: anti–α-COP (883, affinity-purified), 1:1500; anti–β-COP (M3A5, ascites), 1:800; anti–β′-COP (C1PL, affinity-purified), 1:3000; anti–γ-COP (serum), 0.4 μg/ml; anti–ε-COP (affinity-purified), 50 ng/ml; and anti–ζ-COP (affinity-purified), 20 ng/ml.
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28. The constructs encoding wild-type GST-WBP1 (GST-KKLETFKKTN) (*12*), mutant GST-WBP1 (GST-KKLETFSSTN), wild-type E19 (GST-KYKSRRSFIDEKKMP), and mutant E19 (GST-KYKSRRSFIDESSMP) in the pGEX-3X vector were described previously (*5*). All procedures were performed as described (*24, 25*).
29. The CD8 chimeras were constructed by the polymerase chain reaction such that the 165 amino acids of the human CD8 extracellular domain were preserved. Codon 166 was changed to glycine to introduce a unique Apa I restriction site and was followed by a conserved proline, a stop codon, and an Eco RI site. Oligonucleotides encoding the COOH-terminal 34 amino acids of chop24a [RVVLWSFFEALVLVAMTLGQIYYLKR(F/A)(F/A)EVRRVV] (*12*), preceded by an Apa I site and followed by a stop codon and an Eco RI site, were subcloned into the CD8 construct and inserted into the pECE vector [L. Ellis *et al.*, *Cell* **45**, 721 (1986)]. Sequences were verified by DNA sequencing. Transfection of COS-7 cells was performed with Lipofectin and Lipofectamine (Gibco BRL) for immunofluorescence and pulse-chase analysis, respectively. The OKT8 monoclonal antibody to CD8 (Ortho) and fluorescein-conjugated goat antibodies to mouse immunoglobulin (Molecular Probes) were used at a dilution of 1/30 and 1/100, respectively, for immunofluorescence.
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31. We thank T. Söllner, C. Blobel, and M. Craighead for discussions; C. Harter, F. Wieland, and T. Kreis for antibodies; F. Letourneur and P. Cosson for the GST-WBP1 and GST-E19 constructs; S. Ponnambalam and T. Nilsson for the CD8 cDNA construct; and M. Spiess for the pECE vector. Supported by NIH and the Mathers Charitable Foundation (J.E.R.); the Human Frontier Science Program Organization and the Swiss National Science Foundation (K.F.); and the Deutsche Forschungsgemeinschaft (M.V.).

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A Neostriatal Habit Learning System in Humans

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Amnesic patients and nondemented patients with Parkinson's disease were given a probabilistic classification task in which they learned which of two outcomes would occur on each trial, given the particular combination of cues that appeared. Amnesic patients exhibited normal learning of the task but had severely impaired declarative memory for the training episode. In contrast, patients with Parkinson's disease failed to learn the probabilistic classification task, despite having intact memory for the training episode. This double dissociation shows that the limbic-diencephalic regions damaged in amnesia and the neostriatum damaged in Parkinson's disease support separate and parallel learning systems. In humans, the neostriatum (caudate nucleus and putamen) is essential for the gradual, incremental learning of associations that is characteristic of habit learning. The neostriatum is important not just for motor behavior and motor learning but also for acquiring nonmotor dispositions and tendencies that depend on new associations.

Students of brain and behavior have long recognized that double dissociations (*1*) provide the strongest evidence for separating the functions of brain systems. Recent work with experimental animals has dissociated hippocampal and dorsal striatal learning systems (*2*). Rats with lesions of the hippocampus or anatomically related structures were impaired on tasks thought to require spatial, relational memory, but they were intact at tasks of habit learning that require the gradual, incremental learning of associations. Lesions of the dorsal striatum produced the opposite pattern of results.

Evidence for separate memory systems has also been obtained in humans (*3*). For example, amnesic patients are profoundly impaired on conventional tests of declarative (explicit) memory that assess recall and recognition, but they are intact at a variety of nondeclarative (implicit) memory tasks that assess skill learning, simple forms of

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conditioning, and the phenomenon of priming (4). However, the human data, which are based largely on single (one-way) dissociations (5), are subject to an alternate interpretation that has been difficult to discount completely. Namely, there is a single memory system, and in amnesia, some tasks are simply more sensitive at detecting whatever residual memory ability remains (6). In addition, it is not always clear how the memory distinctions proposed in humans relate to findings in experimental animals. In particular, a neostriatal habit learning system like the one identified in rodents (2) has not been demonstrated in humans (7). In the present study, we tested for a double dissociation between declarative memory and habit memory in humans, seeking a parallel to the reports in experimental animals.

We tested 12 amnesic patients who had bilateral damage to the hippocampal formation or diencephalic midline (8), and 20 nondemented patients with Parkinson's disease (PD) (9), which causes neuronal degeneration within the substantia nigra and loss of a major input to the neostriatum. We also tested 15 controls matched to the patient groups with respect to age and education (10).

Two tasks were administered, a task of probabilistic classification learning and a multiple-choice questionnaire (11). In the first task (Fig. 1), individuals learned gradually which of two outcomes would occur on each trial, given the particular combination of cues that appeared. Each cue was independently and probabilistically related to the outcome, and the two outcomes occurred equally often. The probabilistic structure of the task appears to defeat the normal tendency to try to memorize a solution, and individuals can learn without being aware of the information they have acquired. Information about a single trial is not as useful as information accrued across many trials. In this sense, the task is akin to the kind of gradually acquired, habit learning tasks that depend on the dorsal striatum in experimental animals (2). The second task assessed declarative memory for the classification task by means of eight multiple-choice questions about the cues, the layout of the computer screen, and the training episode (four alternatives, chance = 25% correct).

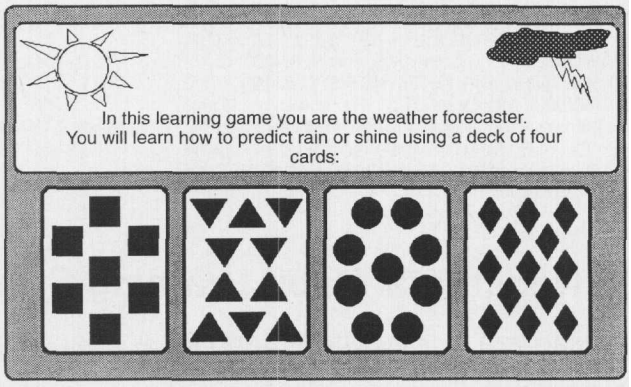

Fig. 1. Appearance of the computer screen at the beginning of the probabilistic classification task. The four cues are shown along with the sun and rain icons. On each trial, one, two, or three of these cues were presented side by side (in 1 of the 14 possible combinations), and individuals predicted whether the outcome would be sunshine or rain by pressing one of two icon keys. Feedback was provided immediately to signal a correct or incorrect response: The appropriate icon appeared above the cues, a high-pitched (correct) or low-pitched (incorrect) tone was sounded, and a vertical bar at the right of the screen increased or decreased by one unit. A particular cue was associated with the outcome sunshine either 75, 57, 43, or 25% of the time, and thus either 25, 43, 57, or 75% of the time with the other outcome (rain). For each person in the study, the four cues were randomly assigned one of these probabilities. Testing proceeded for 50 trials followed by a short break (<1 min) and then for an additional 100 trials with a second break after trial 100.

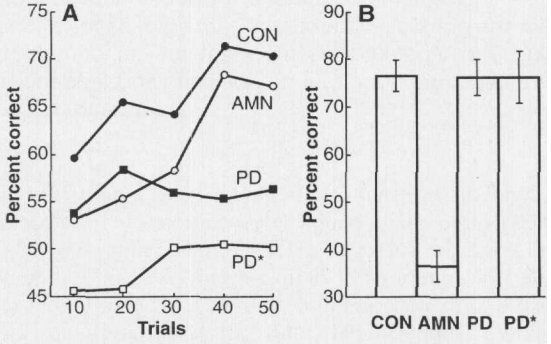

Fig. 2. (**A**) Performance on the probabilistic classification task by controls (CON, $n = 15$), amnesic patients (AMN, $n = 12$), patients with Parkinson's disease (PD, $n = 20$), and a subgroup of the PD patients with the most severe symptoms (PD*, $n = 10$). None of the groups performed significantly above chance levels (50% correct) on the first block of 10 trials. The controls and amnesic patients gradually learned the cue-outcome associations during 50 trials. Standard errors of the mean ranged from 3.8 to 5.8%. A two-way ANOVA (CON compared with AMN) revealed an effect of trial block [analysis of linear trend, $F(1,25) = 10.6$, $P < 0.01$], but no group effect or group x trial block interaction (F values < 1.7, P values > 0.20). The PD patients exhibited no measurable learning across 50 trials (linear, $F < 1.0$), and their score for trials 41 through 50 was no better than chance [56.3%, $t(19) = 1.1$, $P > 0.10$]. Standard errors of the mean for the two PD groups ranged from 4.5 to 8.1%. A two-way ANOVA (CON compared with PD) revealed an effect of group, $F(1,33) = 4.9$, $P < 0.05$. In addition, a comparison of AMN with PD* revealed a significant group x trial block interaction [linear $F(1,30) = 4.2$, $P < 0.05$]. (**B**) Performance on the declarative memory task. Both PD and PD* groups exhibited entirely normal declarative memory for facts about the testing episode, despite their poor performance on the task itself. In contrast, the amnesic patients exhibited a severe impairment in declarative memory for the testing episode but normal performance on the classification test. Brackets show standard errors of the mean.

Across 50 training trials, the amnesic patients learned the classification task as well as controls (Fig. 2A). Both groups began near 50% correct (the score that would be achieved by guessing) and reached a level of about 70% correct in trials 41 through 50 (12). In contrast, the PD patients did not learn the task (Fig. 2A). Impaired learning was particularly evident in the 10 PD patients with the most severe symptoms [Hoehn and Yahr Scale score was ≥3 (9)]. Across the five trial blocks (trials 1 through 50), these 10 patients performed worse than both the control group [analysis of variance (ANOVA), $F(1,23) = 11.4$, $P < 0.01$] and the amnesic patients [$F(1,20) = 5.0$, $P < 0.04$]. In addition, their score in the fifth trial block (trials 41 through 50) was at chance (50.2% correct), worse than the corresponding score of the controls [Student's t test, $t(23) = 2.70$, $P < 0.01$], and marginally worse than the score of the amnesic patients [$t(20) = 1.92$, $P < 0.07$].

In contrast, the PD patients, including the subgroup with the most severe symptoms, performed entirely normally on the test that assessed declarative memory for the classification task (t values < 1.0, Fig. 2B). The amnesic patients, however, performed more poorly than each of the other groups (t values > 6.0, P values < 0.001). Together these results demonstrate a double dissociation of memory function between the brain structures damaged in amnesia (13) and the brain structures damaged in Parkinson's disease. Probabilistic classification learning depends on the neostriatum but not on the medial temporal lobe or diencephalon, and the opposite is the case for declarative memory.

With training extended for an additional 100 trials, the PD patients gradually improved their performance, achieving a score of 60.9 ± 3.1% correct for trials 51 through 100 and 61.9 ± 3.7% for trials 101 through 150 (14). The amnesic patients achieved a

score of 59.2 ± 4.5% correct during trials 101 through 150, and controls scored 66.1 ± 2.5% correct (*15*). Continued training may allow information to become available from declarative memory, that is, the controls and the PD patients may have eventually detected and memorized some of the cue-outcome associations (*11*).

The impaired performance of PD patients on the probabilistic classification task parallels the habit learning deficits observed in experimental animals with lesions of the dorsal striatum. However, because Parkinson's disease is thought to affect the function of the frontal lobes (*16*), and because our PD patients had neuropsychological signs of frontal lobe dysfunction (*17*), we considered the possibility that frontal lobe dysfunction, rather than neostriatal dysfunction, could explain the impairment we observed. We therefore tested 10 patients with circumscribed damage to the frontal lobes (*18*). These patients obtained scores of 53.9, 68.0, 63.1, 54.6, and 58.2% on the first five trial blocks (trials 1 through 50). Their overall score of 60.5 ± 5.0% correct for the first 50 trials was similar to the scores of the amnesic and control groups (58.5 ± 3.3% and 65.9 ± 3.3% correct, respectively, P values > 0.10), and marginally better than the score of the 10 severely affected PD patients [$t(18) = 2.0$, $P = 0.06$] (*19*). On the questionnaire, the frontal patients obtained a normal score of 68.1 ± 4.7% correct.

Whereas some neuropsychological findings have emphasized the similarities between Parkinson's disease and frontal lobe pathology (*7*, *20*), our findings show that the cognitive impairments can be distinguished (*21*). Not only did the frontal patients improve at the classification task more quickly than the severely affected PD patients (*19*), but performance on the classification task correlated with the symptoms of Parkinson's disease and not with symptoms of frontal lobe dysfunction (*22*).

In humans the neostriatum (caudate nucleus and putamen) appears to be part of a brain system supporting the gradual, incremental learning characteristic of habit learning. Synaptic changes responsible for habit learning could occur in the neocortex, such that cortical targets in the neostriatum receive altered input. This could result in the creation and selection of new dispositions and actions for behavior. Alternatively, learning could occur in the neostriatum itself, as changes within corticostriatal projections. Other memory systems depend on limbic-diencephalic and neocortical interaction (declarative memory), the amygdala and neocortex (emotional conditioning), the cerebellum (classical conditioning of skeletal musculature), and the posterior neocortex (perceptual priming) (*3*).

Previous studies of patients with basal ganglia disorders, such as Huntington's disease and Parkinson's disease, have documented learning impairments in procedural tasks, notably, those that require the generation of motor programs (*23*). The present findings show that a neostriatal system in humans is important not just for motor learning but also for acquiring nonmotor habits that depend on new associations (*24*). These nonmotor habits presumably include a wide range of dispositions and tendencies, which are shaped by reward, specific to particular stimuli, and which guide behavior and cognition.

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9. The diagnosis of Parkinson's disease (17 men and 3 women) was confirmed by a senior staff neurologist at the University of California Medical Center, San Diego. The patients averaged 65.2 years of age (range, 46 to 79) and an average of 16.0 years of education. They scored 54.2 and 25.1 on the vocabulary and information subscales, respectively, of the WAIS-R [compare with (*8*, *10*)]. Their mean score on the Dementia Rating Scale was 137.7, indicating an absence of dementing illness [maximum score = 144, S. Mattis, in *Geriatric Psychiatry*, R. Bellack and B. Keraso, Eds. (Grune and Stratton, New York, 1976)]. The mean severity of Parkinsonian symptoms was stage 2.8 as rated by the Hoehn and Yahr Scale, 1 = least severe, 5 = most severe [M. M. Hoehn and M. D. Yahr, *Neurology* **17**, 427 (1967)], and was 10.5 as rated by the Unified Parkinson's Disease Rating Scale, hand and foot subscale, 0 = normal, 32 = most severe [S. Fahn *et al.*, in *Recent Developments in Parkinson's Disease*, S. Fahn, C. D. Marsden, M. Goldstein, D. B. Calne, Eds. (Macmillan, New York, 1987)]. The mean score on the Beck Depression Inventory was 5.4 (maximum = 63), indicating an absence of clinical depression [A. T. Beck, C. H. Ward, M. Mendelson, J. Mock, I. Erbaugh, *Arch. Gen. Psychiatry* **56**, 561 (1961)]. At the time of testing, all patients were under the care of a neurologist and were optimally medicated. All of the patients were receiving dopamine precursor treatment (Sinemet). In addition, 12 patients were taking a monoamine oxidase inhibitor (Eldepryl), 8 were taking a dopamine enhancing drug (Parlodel, Permax, or Amantadine), and 3 were taking an anticholinergic drug (Artane). Removing the latter three patients did not noticeably affect the results.
10. The controls (seven men and eight women) averaged 65.1 years of age (range, 54 to 77) and 14.2 years of education. They scored 54.0 and 20.9 on the vocabulary and information subscales of the WAIS-R, respectively.
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18. The patients with frontal lobe lesions (six men and four women) averaged 68 years of age (range, 62 to 76 years) and 13.7 years of education. Six had sustained left frontal lesions, three had sustained right frontal lesions, and one had bilateral frontal lobe lesions. On the WCST (*17*), they achieved 3.6 categories and made 32.8% perseverative errors, marginally more than the Parkinson patients, $t(28) = 1.76$, $P = 0.09$. For reconstructions of the frontal lesions and examples of their impaired performance on other tests, see J. S. Janowsky, A. P Shimamura, M. Kritchevsky, L. R. Squire, *Behav. Neurosci.* **103**, 548 (1989) (patients JD, MD, JV); A. P. Shimamura, P. J. Jurica, J. A. Mangels, F. B. Gershberg, R. T. Knight, *J. Cognit. Neurosci.* **7**, 144 (1995) (patients EB, AL, MM, RM); J. A. Mangels, F. B. Gershberg, A. P. Shimamura, R. T. Knight, *Neuropsychology* **10**, 32 (1996) (patients OA, JD); L. L. Chao and R. T. Knight, *Neuroreport* **6**, 1605 (1995) (patient JC).
19. In addition, analysis of variance (severe PD patients compared with frontal patients and three 50-trial blocks: trials 1 through 50, 51 through 100, and 101 through 150) revealed a significant group x trial block interaction [$F(2,34) = 3.52$, $P < 0.04$]. The interaction resulted from the fact that the frontal patients, like the amnesic patients and controls, scored about the same overall in each of the three 50-trial blocks (frontals: 60.5, 56.5, and 66.1%; amnesics: 58.5, 61.2, and 59.2%; controls: 65.9, 67.3, and 66.1%). In contrast, the severely affected PD patients scored 47.8% correct (at chance) on trials 1 through 50, 58.2% on trials 51 through 100, and 61.1% on trials 101 through 150.
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21. Also see A. M. Owen *et al.*, *Brain* **116**, 1159 (1993); A. M. Owen *et al.*, *Neuropsychology* **9**, 126 (1995). Despite these differences between the effects on cognition of frontal lobe lesions and Parkinson's disease, the cognitive impairment in Parkinson's disease presumably arises from the effect that neostriatal lesions exert on the targets of the basal ganglia, which include frontal cortex.
22. The PD patients who performed poorest on the classification task (percent correct score during trials 1 through 50) also obtained the highest Hoehn and Yahr Scale scores of symptom severity (*9*) correlation coefficient, $r = -0.55$, $P < 0.02$). In contrast, in the case of the frontal patients, performance on the first 50 trials of the classification test was slightly and nonsignificantly better for those patients with the most severe frontal symptoms [measured by the number of categories achieved correctly on the WCST (*17*) and by the percent perseverative error score, $r = -0.14$ and $r = 0.22$, respectively]. Interestingly, for the PD patients, poor classification learning also correlated with frontal lobe symptoms ($r = 0.63$, $P < 0.01$ for categories; $r = -0.69$, $P < 0.01$ for perseverative errors), as would be expected if increasing frontal lobe dysfunction reflects the progression of the primary disease in the neostriatum. However, the overall pattern of correlations suggests that the neostriatal symptoms, not the frontal lobe symptoms, best predicted classification learning. This conclusion depends on the assumption that frontal lobe dysfunction in the PD patients was no more severe than in the patients with frontal lobe lesions. The neuropsychological findings (*17*, *18*) are consistent with this idea.
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24. Patients with Huntington's disease also did not learn the probabilistic classification task (B. J. Knowlton *et al.*, *Neuropsychology*, in press). However, it is difficult to isolate this deficit to the basal ganglia because of the dementia and widespread neuropathology associated with Huntington's disease.
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A Requirement for Local Protein Synthesis in Neurotrophin-Induced Hippocampal Synaptic Plasticity

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Two neurotrophic factors, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), are able to produce a long-lasting enhancement of synaptic transmission in the hippocampus. Unlike other forms of plasticity, neurotrophin-induced plasticity exhibited an immediate requirement for protein synthesis. Plasticity in rat hippocampal slices in which the synaptic neuropil was isolated from the principal cell bodies also required early protein synthesis. Thus, the neurotrophins may stimulate the synthesis of proteins in either axonal or dendritic compartments, allowing synapses to exert local control over the complement of proteins expressed at individual synaptic sites.

The cellular changes that underlie both synaptic and behavioral plasticity are usually classified as either (i) short term, because they are based on the modification of preexisting proteins, or (ii) long term, because they require protein synthesis. For example, studies of synaptic plasticity in the hippocampus and in *Aplysia* have shown that, whereas the short-term phase (0 to 1 hour) of synaptic enhancement is not blocked by inhibitors of protein translation, the long-term phase (>1 hour) is [(*1*), but see (*2*)]. These cellular studies are paralleled by many studies of behavioral plasticity that also indicate that short-term memories are insensitive to inhibitors of protein synthesis (*3*). The neurotrophic factors BDNF and NT-3 can enhance synaptic efficacy (*4*), and we have now examined the temporal sensitivity of the neurotrophin-induced synaptic enhancement to inhibitors of protein synthesis.

Synaptic transmission was examined at the Schaffer collateral–CA1 pyramidal neuron synapse in adult rat hippocampal slices with the use of conventional extracellular recording techniques (*5*). In control experiments, extracellular application of BDNF (50 ng/ml) or NT-3 (50 ng/ml) elicited a robust enhancement of synaptic transmission (Fig. 1, A and B) (*4*) [mean percent of baseline: BDNF, 221.4 ± 16.4 (mean ± SEM, n = 7), $P < 0.005$; NT-3, 231.1 ± 19.5 (n = 8), $P < 0.005$]. Pretreatment with one of two protein synthesis inhibitors (*6*), either anisomycin (40 μM) or cycloheximide (40 μM), markedly attenuated the synaptic enhancement induced by either neurotrophin (Fig. 1, C through F and H) [mean percent of baseline: BDNF plus anisomycin, 134.2 ± 8.4 (n = 9), $P < 0.05$; BDNF plus cycloheximide, 138.7 ± 13.2 (n = 7), $P < 0.05$; NT-3 plus anisomycin, 130.1 ± 7.6 (n = 9), $P < 0.05$; NT-3 plus cycloheximide, 118.5 ± 14.0 (n = 7), not significant (NS)]. In contrast to previous studies of synaptic plasticity, the sensitivity to inhibitors of protein synthesis was evident within minutes of neurotrophin application (Fig. 1, C through F). Similar pretreatment of hippocampal slices with an inhibitor of prokaryotic protein synthesis, chloramphenicol (80 μM), did not significantly reduce the synaptic enhancement

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