11. Hamilton, W. D. *Science* **156,** 477–488 (1967).
12. Vollrath, F. *Z. Tierpsychol.* **53,** 61–78 (1980).
13. Pagel, M. D. & Harvey, P. H. *Q. Rev. Biol.* **63,** 413–440 (1988).
14. Christenson, T. E. in *Contemporary Issues in Comparative Psychology* (ed. Dewsbury, D. A.) 149–174 (Sinaur, Sunderland, Massachusetts, 1990).
15. Vollrath, F. in *Ecophysiology of Spiders* (ed. Nentwig, W.) 357–370 (Springer, Berlin, 1987).
16. Newman, J. A. & Elgar, M. A. *Am. Nat.* **138,** 1372–1395 (1991).
17. Gertsch, W. J. *American Spiders* (Van Nostrad, New York, 1949).
18. Levi, H. W. *J. New York ent. Soc.* **58,** 59–91 (1955).
19. Watson, P. J. *Behavl Ecol. Sociobiol.* **26,** 77–90 (1990).
20. Gunnarson, B. *J. Zool.* **217,** 1–7 (1989).
21. Clutton-Brock, T. *The Evolution of Parental Care* (Princeton University Press, 1991).
22. Emlen, S. T. & Oring, L. W. *Science* **197,** 215–223 (1977).
23. Parker, G. A. *J. Fish. Biol.* (in the press).
24. Maynard Smith, J. *Evolution and the Theory of Games* (Cambridge University Press, 1982).
25. Clutton-Brock, T. & Parker, G. A. *Q. Rev. Biol.* (in the press).
26. Smith, G. *Fauna Flora Golf. Neapel* **29,** 1–123 (1906).
27. Caullery, M. *Mitt. zool. Stn Neapel* **18,** 583–643 (1908).
28. Bertelsen, E. *Dana Rep.* **39,** 1–176 (1951).
29. Beebe, W. *Zoologica* **6,** 149–241 (1934).
30. Lockett, G. H. & Millidge, A. F. *British Spiders* Vol. 1 (Royal Society, London, 1951).
31. Lockett, G. H. & Millindge, A. F. *British Spiders* (Royal Society, London, 1953).
32. Shinkai, E. & Takano, S. *Spiders of Japan* (Tokai University Press, 1984).
33. Koh, J. K. H. *A Guide to Common Singapore Spiders* (Singapore Science Centre, 1989).
34. Mascord, R. *Australian Spiders* (Reed, Wellington, New Zealand, 1970).

ACKNOWLEDGEMENTS. We thank S. Stearns for assistance.

Motor learning in a recurrent network model based on the vestibulo–ocular reflex

S. G. Lisberger* & T. J. Sejnowski†

* Department of Physiology, W. M. Keck Foundation Center for Integrative Neuroscience, and Neuroscience Graduate Program, University of California, San Francisco, California 94143, USA
† Salk Institute for Biological Science, Howard Hughes Medical Institute, 10010 N. Torrey Pines Road, La Jolla, California 92093, and Department of Biology, University of California, San Diego, California 92093, USA

MOST models of neural networks have assumed that neurons process information on a timescale of milliseconds and that the long-term modification of synaptic strengths underlies learning and memory[1]. But neurons also have cellular mechanisms that operate on a timescale of tens or hundreds of milliseconds, such as a gradual rise in firing rate in response to injection of constant current[2] or a rapid rise followed by a slower adaptation[3]. These dynamic properties of neuronal responses are mediated by ion channels that are subject to modulation[4]. We demonstrate here how a neural network with recurrent feedback connections can convert long-term modulation of neural responses that occur over these intermediate timescales into changes in the amplitude of the steady output from the system. This general principle may be relevant to many feedback systems in the brain. Here it is applied to the vestibulo–ocular reflex, whose amplitude is subject to long-term adaptive modification by visual inputs[5]. The model reconciles apparently contradictory data on the neural locus of the cellular mechanisms that mediate this simple form of learning and memory.

We used model neurons in which the relationship between the summed inputs (V_{in}) and the firing rate output (V_{out}) was described by a single time constant (τ) which determined the rate at which V_{out} would rise in response to a stimulus increasing from zero to V_{in}. The input (V_{in}) to the model neuron was in the form of a brief ramp from zero to one unit and caused an output that rose more gradually from zero to one. The rising phase of the model neuron's output was a smoothed version of the input waveform when the time constant was 20 ms and a slower and smoothed version of the waveform when the time constant was 70 ms (Fig. 1). It is important to emphasize that the time constant in our model neurons is not equivalent to the membrane time constant; rather it describes the time course of changes in firing rate for a step change in input current and, in real neurons, it would be determined by the combined properties of a number of cellular mechanisms.

Figure 2*a* shows an example of a model network of neurons that can convert the small and transient difference between the two outputs of the model neuron in Fig. 1 into a large and sustained change in the steady-state output from the system. The input to the network is V and the output is E. The time courses of the responses of units T and F were determined by single time constants, like the model neuron in Fig. 1. Units P and B did not have time constants and their outputs at each time were equal to the sum of their inputs at the time. The strength of transmission of signals from one unit to the next was regulated by multiplication factors that scaled each input. For example, W_P scales the inputs from unit T to unit P and W_B scales the input from V to unit B. Other multiplication factors had values of +1 for excitatory connections and −1 for inhibitory connections.

Figure 2*b* shows how the network in Fig. 2*a* responded before and after the time constant of unit T was changed. To obtain the traces labelled 'Before', the values of W_B and W_P were both 1 and the time constants were 70 ms in units T and F. The input applied (V) then caused an identical output (E): the gain of the system, defined as E/V, was 1. When the time constant of unit T was decreased from 70 ms (Before) to 20 ms (traces labelled 'After'), the output of the network (E) was smaller and unit P showed a large response to the input waveform. Thus, changes in the time course of the response of unit T cause the steady-state gain of the system to be reduced, even though no changes were made to the scaling factors in the model.

Recurrent connections through units P, B, and F are the critical design feature that determines how the model works. These connections form a positive-feedback loop that allows a change in the time course of the output from one unit to be converted to a change in steady-state gain of the system. This can be understood intuitively by realizing that positive feedback will perform mathematical integration in a model that processes time-varying inputs. When the time constants in units T and F are the same and the values of W_P and W_B are 1, the two inputs to unit P cancel each other at all times and there is no input to be integrated. When the time constants in units T and F are

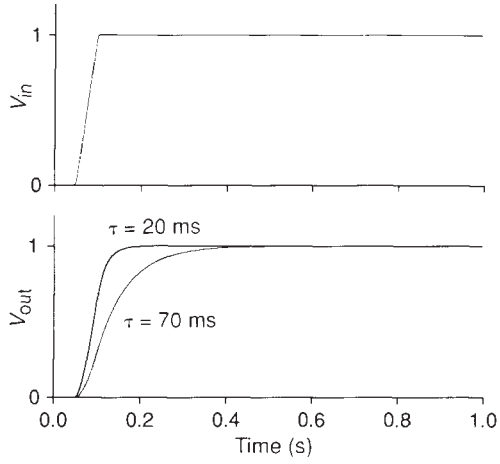

FIG. 1 Time-course of response of a simple model neuron in which a step input is transformed into an exponential rise in firing rate. The input (V_{in}) and output (V_{out}) of the unit are plotted as a function of time, showing the output when the time constant of the unit is 20 and 70 ms.
METHODS. Simulations were conducted with 'A Simulation Package', written by L. Optican and H. Goldstein. The models were described as block diagrams and the dynamic units were represented in Laplace notation as $1/(s\tau+1)$, where τ is the time constant of filtering and s is the Laplace operator. The block diagrams were described in a Block Oriented Modeling Language (BOMOL) and a lexical analyser was used to convert the BOMOL code into the 'C' programming language. The program was then compiled and linked into a simulation shell that allowed control over the values of the parameters of the model and provided stimulus generation and graphical display of outputs. The time-step of simulation was 1 ms, much shorter than any of the time constants used here.

different, the two inputs to unit P differ transiently. This injects a transient into the positive-feedback loop. The transient is integrated and remembered and is expressed as a steady-state decrease in the output from the model.

The model network in Fig. 2*a* suggests a general algorithm for learning and may be applicable to the operation of many brain pathways that include recurrent connections. The network was selected for our studies specifically because of its relevance to the neural pathways that generate the vestibulo-ocular reflex (VOR)[6]. The VOR uses vestibular inputs to the brainstem and cerebellum to generate smooth eye movements that help stabilize images on the retina during head turns[5]. In Fig. 2, unit P represents a specific group of Purkinje cells in the cerebellum[6] and unit B represents neurons in the brainstem[7]. The model must contain a positive-feedback pathway through units B, F and P because of evidence that such a recurrent pathway exists in the brain[8]. In the brain, the positive-feedback pathway carries an efference copy of eye velocity commands back to the cerebellum and is important in the generation of visually guided smooth pursuit eye movements. During pursuit, the positive-feedback pathway is used to integrate transient visual motion inputs into a sustained eye velocity output[9].

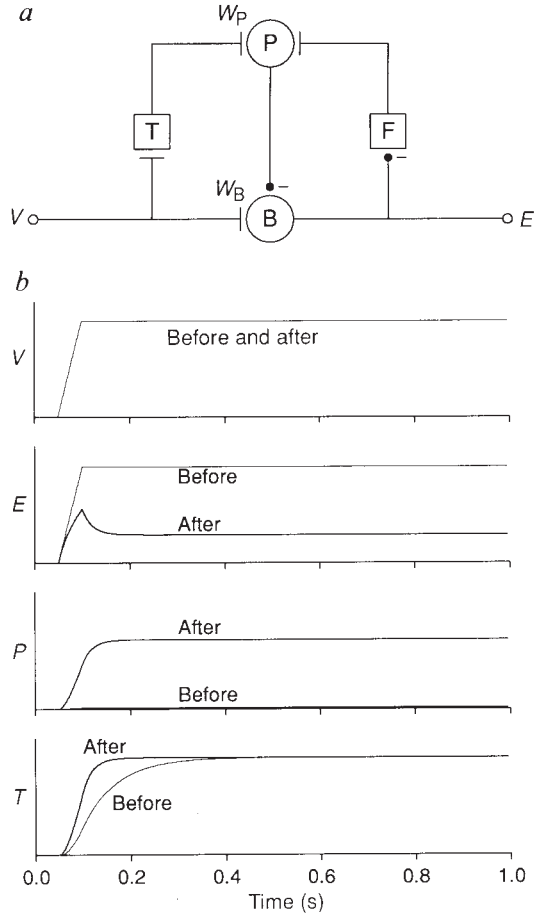

FIG. 2 Performance of a recurrent network that is based on the flow of signals in the primate vestibulo-ocular reflex. *a*, Diagram of the network, which includes a feedback loop with positive gain. Units T and F have the properties described in Fig. 1 and P and B operate as simple addition units without dynamical properties. Initially, units T and F both had time constants of 70 ms. W_P and W_B indicate the weights of the connections from unit T to unit P and from V to unit B, respectively. The connections indicated by minus signs have weights of −1 and the other connections have weights of +1. *b*, Effect of changing the time constant of unit T from 70 ms (Before) to 20 ms (After). Even though this did not change the steady-state response of unit T, it caused a profound reduction in the steady-state output from the network and a pronounced increase in the response of unit P. The steady-state gain of the system was 1.0 when τ_T was 70 ms and was 0.28 when τ_T was 20 ms. In applying the model to the VOR, unit P represented the 'horizontal gaze-velocity Purkinje cells' in the flocculus and ventral paraflocculus of the cerebellum[5,7,8,14] and unit B represented the 'flocculus target neurons' in the vestibular nuclei[7].

The gain of the VOR, defined as eye speed divided by head speed, is normally near 1, even in darkness when there is no possibility of visual correction[10]. The excellent performance of the VOR is established and maintained by a learning mechanism that uses the association of visual and vestibular inputs to guide adaptive changes in the gain of the VOR[11,12]. Previous hypotheses have suggested that learning is mediated by changes in the gain of steady-state transmission at different synaptic relays but have disagreed about the loci of learning. Recordings under some conditions[6,14] have been consistent with the suggestion[13] that decreases in the gain of the VOR are mediated by increases in the value of W_P. Decreases in the gain of the VOR caused the output of the Purkinje cells, represented by unit P, to become modulated in phase with their vestibular inputs during the VOR. In contrast, recordings under other conditions[6,7] appeared to contradict the hypothesis[13]: decreases in the gain of the VOR were associated with decreases in the value of W_P. It was postulated that decreases in the gain of the VOR would be mediated by parallel decreases in the value of W_B and W_P[6].

When applied to the VOR, the model in Fig. 2*a* offers a way to resolve the controversy between these two hypotheses as well as the apparently contradictory data[6,14]. Analytical solution of the model in Fig. 2*a* shows that the model is stable only if W_B and W_P have the same value. Under these conditions, the gain of the VOR will be $W_P(\tau_T/\tau_F)$, where τ_T and τ_F are the time constants of units T and F, respectively. It was found that the neural equivalent of W_P was reduced to 81% of normal when the gain of the VOR was 0.18. Therefore, the solution of our model predicts that τ_T/τ_F should be 0.22. If we assume further that τ_F is fixed at 70 ms, then τ_T should be reduced to 18 ms. The analytical solution also reveals that the steady-state output of unit P during the VOR will be equal to $VW_P(\tau_F-\tau_T)/\tau_F$. Thus, the model predicts that the output of unit P will be in phase with vestibular input V when the gain of the VOR is low, as found by experiment[6,7,14].

Application of the model in Fig. 2*a* to the VOR therefore simulates motor learning in the VOR without contradicting any available data[15]. In addition, the output from the model (E in Fig. 2*b*) reproduces the observation that reductions in the gain of the VOR cause the eye velocity evoked by rapid head turns to show a transient overshoot before settling to a steady, sustained level[16]. In the model, motor learning is accomplished by several mechanisms at two or more loci. This suggests that learning in monkeys may be effected by a combination of changes in steady-state synaptic transmission in the brain stem and cerebellum and changes in time-course of the vestibular inputs to the cerebellum.

We described the time course of the response of model neurons to a step input with a single time constant so that we could both simulate (Fig. 2*b*) and calculate the performance of the network in Fig. 2*a*. Our simulations showed that changes in the transient component of a neuron's responses can be transformed into changes in the steady-state output from a network. Although a more complicated model would be required to describe the time-course of the responses of real neurons to a step increase in input current, the basic effects of changes in the transient response would be the same as those revealed here. In the brain, changes in the dynamics of neuronal responses could result from modulation of intrinsic cellular mechanisms, modulation of the strengths of local feedback connections, or changes in the selection of inputs with different dynamics. This third possibility is especially intriguing in a sensory-motor system like the VOR. In the vestibular system, some afferents respond to a head velocity stimulus with a tonic change in firing rate while others have a phasic/tonic response[16]. The mechanism demonstrated here could reduce the steady-state gain of the VOR by using local learning mechanisms to increase the

influence of the vestibular inputs that transmit phasic/tonic signals to the cerebellum while decreasing the influence of the inputs that transmit purely tonic signals[15].

The recurrent positive feedback pathway in our neural network was critical for converting a subtle change in the time-course of neuronal responses into a change in the steady-state output of the system. Feedback connections are a general architectural feature of the brain and are found at many different levels, including the inputs and outputs of the cerebellum and the connections between areas of the cerebral cortex. The importance of recurrent connections in the pathways that mediate the VOR[9] allows us to model how recurrent connections could contribute to learning and memory. The model raises the possibility that subtle changes in the function of individual cellular mechanisms may have profound effects on the output from specific behavioural systems and emphasizes the importance of understanding the architecture of the neural networks that convert cellular changes into changes in behavioural output. □

Received 5 May; accepted 18 September 1992.

1. Churchland, P. S. & Sejnowski, T. J. *The Computational Brain* (MIT Press, Cambridge, MA, 1992).
2. Storm, J. F. *Nature* **336,** 379–381 (1988).
3. Bradley, K. & Somjen, G. G. *J. Physiol., Lond.* **156,** 75–92 (1961).
4. Strong, J. A. & Kaczmarek, L. K. in *Neuromodulation: The Biochemical Control of Neuronal Excitability* (eds Kaczmarek, L. K. & Levitan, I. B.) (Oxford University Press, 1987).
5. Lisberger, S. G. *Science* **242,** 728–735 (1988).
6. Miles, F. A., Braitman, D. J. & Dow, B. M. *J. Neurophysiol.* **43,** 1477–1493 (1980).
7. Lisberger, S. G. & Pavelko, T. A. *Science* **242,** 771–773 (1988).
8. Miles, F. A., Fuller, J. H., Braitman, D. J. & Dow, B. M. *J. Neurophysiol.* **43,** 1437–1476 (1980).
9. Lisberger, S. G., Morris, E. J. & Tychsen, L. *A. Rev. Neurosci.* **10,** 97–129 (1987).
10. Fuchs, A. F. & Kimm, J. *J. Neurophysiol.* **38,** 1140–1161 (1975).
11. Gonshor, A. & Melvill Jones, G. *J. Physiol., Lond.* **256,** 381–414 (1976).
12. Miles, F. A. & Lisberger, S. G. *A. Rev. Neurosci.* **4,** 273–299 (1981).
13. Ito, M. *Brain Res.* **40,** 81–84 (1972).
14. Watanabe, E. *Brain Res.* **297,** 169–174 (1984).
15. Lisberger, S. G. & Sejnowski, T. J. *Univ. California San Diego Inst. Neural Comput. Tech. Rep.* 9201 (1992).
16. Lisberger, S. G. & Pavelko, T. A. *J. Neurosci.* **6,** 346–354 (1986).

ACKNOWLEDGEMENTS. We thank numerous colleagues for their helpful comments. Research was supported by a grant from the Defense Advanced Research Project Agency, awarded through the Office of Naval Research.

A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT$_{1B}$ receptors

Donna Oksenberg*†, Scot A. Marsters*, Brian F. O'Dowd‡, Hui Jin‡, Sona Havlik†, Stephen J. Peroutka‡§ & Avi Ashkenazi*

Departments of * Pulmonary Research and Gene Therapy, and § Neuroscience, Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA
† Department of Neurology, Stanford University School of Medicine, Stanford, California 94305, USA
‡ Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

NEUROPSYCHIATRIC disorders such as anxiety, depression, migraine, vasospasm and epilepsy may involve different subtypes of the 5-hydroxytryptamine (5-HT) receptor[1,2]. The 1B subtype, which has a unique pharmacology, was first identified in rodent brain[3–7]. But a similar receptor could not be detected in human brain[6], suggesting the absence in man of a receptor with equivalent function. Recently a human receptor gene was isolated (designated 5-HT$_{1B}$ receptor[8,9], 5-HT$_{1D\beta}$ receptor[10,11], or S12 receptor[12]) which shares 93% identity of the deduced protein sequence with rodent 5-HT$_{1B}$ receptors[13–15]. Although this receptor is identical to rodent 5-HT$_{1B}$ receptors in binding to 5-HT, it differs profoundly in binding to many drugs. Here we show that replacement of a single amino acid in the human receptor (threonine at residue 355) with a corresponding asparagine found in rodent 5-HT$_{1B}$ receptors renders the pharmacology of the receptors essentially identical. This demonstrates that the human gene does indeed encode a 1B receptor, which is likely to have the same biological functions as the rodent 5-HT$_{1B}$ receptor. In addition, these findings show that minute sequence differences between homologues of the same receptor from different species can cause large pharmacological variation. Thus, drug–receptor interactions should not be extrapolated from animal to human species without verification.

TABLE 1 Ligand-binding properties of the wild-type and T355N mutant human receptors compared with those of the rat and mouse 5-HT$_{1B}$ receptors

Ligand	K_i (nM)			
	Human (wild type)	Human (T355N)	Rat[14] (5-HT$_{1B}$)	Mouse[15] (5-HT$_{1B}$)
Serotonergic				
5-HT	10 ± 1	8 ± 1	16 ± 1	39
5-CT	4 ± 1	3 ± 1	7 ± 1	10
DHE	6 ± 1	2 ± 1	4 ± 2	NA
RU24969	44 ± 4	2 ± 1	2 ± 1	10
Metergoline	25 ± 3	200 ± 40	129 ± 33	NA
Sumatriptan	38 ± 3	560 ± 100	465 ± 85	NA
Methysergide	130 ± 7	970 ± 130	1,823 ± 297	NA
8-OH-DPAT	1,600 ± 100	25,000 ± 1,000	>10,000	30,000
Methiothepin	12 ± 1	38 ± 8	13 ± 4	NA
β-adrenergic				
(−)Propranolol	8,100 ± 400	17 ± 1	57 ± 4	NA
(−)Pindolol	11,000 ± 1,000	20 ± 3	153 ± 62	69
(−)Alprenolol	11,000 ± 800	13 ± 1	NA	NA

The values are depicted as mean ± s.e.m. from 4 (wild type) and 3 (T355N) independent experiments done in triplicates. NA, data not available. Complementary DNAs encoding the wild-type and T355N mutant human receptors were inserted into the mammalian expression vector pRK5 and introduced by transient transfection into the human embryonic kidney 293 cell line by a modified calcium phosphate precipitation method[23]. The cells were collected by centrifugation 48 h after transfection, lysed in ice-cold buffer (50 mM Tris-HCl, pH 7.4, containing 5 mM EDTA), homogenized, and sonicated for 10 s. Nuclei and intact cells were removed by centrifugation at 1,000*g* for 10 min. The supernatant was spun at 35,000*g* for 30 min and the resulting pellet, containing the microsomal membrane fraction, was resuspended in binding buffer containing 50 mM Tris-HCl (pH 7.4), 4 mM $CaCl_2$, 0.1% ascrobic acid, 10 mM pargyline and 1 μM leupeptine. Microsomal membranes (50 μg protein) were incubated with the ligands in binding buffer (30 min, 25 °C). Binding was terminated by the addition of 5 ml ice-cold 50 mM Tris-HCl (pH 7.8), rapid vacuum filtration through glass fibre filters, and two subsequent 5-ml washes. Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M cold 5-HT. Scatchard analyses of saturation binding of [^{3}H]5-HT showed two populations of binding sites; equilibrium dissociation constants (K_D) for the wild-type and mutant receptor, respectively, were 4.6 ± 1.4 and 3.9 ± 1.2 nM (high-affinity sites); 72 ± 24 and 75 ± 14 nM (low-affinity sites). The respective receptor densities in pmol per gram of protein were 1,000 ± 320 and 1,440 ± 610 (high-affinity sites), 13,670 ± 1,860 and 25,330 ± 4,480 (low-affinity sites). Specific binding of [^{3}H]5-HT was not detectable in untransfected cells (not shown), indicating that these cells do not express significant levels of endogenous 5-HT receptors. Equilibrium inhibition constants (K_i) were determined according to the following equation: $K_i = IC_{50}/(1 + [T]/K_D)$, where IC_{50} is the concentration of competing ligand required for 50% inhibition of [^{3}H]5-HT binding, [T] is the concentration of the [^{3}H]5-HT tracer (3 nM), and K_D is the high-affinity constant of [^{3}H]5-HT, as determined by saturation binding. The data were analysed by nonlinear least-square fitting using the EBDA[24] and LIGAND[25] programs.

The human and rodent 5-HT$_{1B}$ receptors bind to 5-HT with comparably high affinity. But the human receptor binds with much lower affinity to the serotonergic agonist RU 24969 and to the β-adrenergic receptor antagonists propranolol, pindolol and alprenolol, and with higher affinity to several serotonergic drugs, including sumatriptan and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)[8–14] (Table 1).

The 32 amino-acid differences between the human and rat receptors are scattered throughout the molecule, but only eight are found in the transmembrane domains, which are thought to contain the ligand-binding pocket (Fig. 1). An asparagine residue in the seventh transmembrane segment has been implicated in β-antagonist binding to the β-adrenergic receptor[16] and to human 5-HT$_{1A}$ receptors[17]. Notably, an asparagine