corresponding border within Brodmann's area 4 separated areas with differing densities of [^{3}H]ketanserin-binding sites[14] (mainly serotoninergic 5-HT$_2$ receptors; data not shown). The images of the sections of both cytoarchitectonic and autoradiographic studies were transformed into standard anatomical format by the computerized Human Brain Atlas[14–17] so that structural findings could be compared with those of the positron emission tomography (PET) studies. An overlay map was calculated and area 4a and 4p were defined as the space of the brain within which area 4a or 4p could be found in at least three of five brains (Fig. 3*a*).

To test whether the two subareas differed functionally we analysed PET activations of M1 in four groups of normal right-handed subjects performing different motor actions with the fingers of the right hand (Fig. 3). The hypotheses were (1) that double representations of the fingers might exist (as suggested in refs 6, 7) and (2) that externally triggered movements and movements guided by somatosensory information might activate area 4a and 4p differentially. The PET images were transformed into the standard format of the computerized brain atlas, as were the images of the structural studies. We then checked whether the locations of the PET activations were within areas 4a or 4p (Fig. 3).

In reaction-time tasks, subjects pressed a response key with their right thumb. The somatosensory reaction-time task (response to touch on right index finger) activated two separate fields within M1, one located medially within area 4p, and one more superiorly and laterally within 4a (Fig. 3*b*). In this task, the movement, a thumb flexion to press the key, was not guided by the tactile stimulus, in the sense that it did not provide any information about how the thumb flexion should be performed. In a visual reaction-time task, activation of areas 4a and 4p overlapped the active regions of the somatosensory reaction-time task (Fig 3*c*,*f*). This indicates that two different representations of the thumb might exist. In a tactile discrimination task the subjects examined ellipsoids with complex, learned, stereotypical movements in which mainly the thumb, index and middle finger were active[18–20]. The trajectories of the fingers were guided by the curvatures of the objects[18,19]. Although most of the activation was localized to area 4p, overlapping the activations of the reaction-time tasks (Fig. 3*c*), a separate overlap was also found between the 4a reaction-time task activation and the tactile discrimination task (Fig. 3*f*). The probability that such activations from two independent studies by chance should overlap both in area 4a and in area 4p was very small. This seems to confirm that two different representations of the thumb exist.

In a visual memory task (Fig. 3, legend), the subjects used the index and middle finger for responses. This activated area 4a in regions overlapping the tactile discrimination activation but not those associated with the reaction-time tasks (Fig. 3*d*, *e*). Finally, in a roughness discrimination task, the subjects using the thumb and index finger showed activation in area 4p in almost exactly the same territory as in the tactile discrimination task (Fig. 3*c*). This indicates that also two representations of the index finger and presumably also the middle finger exist: one within area 4a and one within area 4p. A clue to a possible separation of function arose from the observation that roughness discrimination did not activate area 4a, but activated area 4p significantly more than the control condition of self-generated movements ($P < 0.05$).

We have described two structurally different areas in the primary motor cortex, 4a and 4p, each having one representation of the thumb, index and possibly middle finger. These areas differ cytoarchitecturally, neurochemically, and possibly functionally, although a double dissociation of function has not been shown. □

Received 20 May; accepted 1 July 1996.

1. Fritsch, G. & Hitzig, E. *Arch. Anat. Physiol. Wiss. Med.* **37,** 300–332 (1870).
2. Foerster, O. in *Handbuch der Neurologie* (eds Bumke, O. & Foerster, O.) 1–357 (Springer, Berlin, 1936).
3. Penfield, W. & Boldrey, E. *Brain* **60,** 389–443 (1937).
4. Woolsey, C. N. *et al. Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **30,** 238–264 (1952).
5. Brodmann, K. *Vergleichende Lokalisationslehre der Grosshirnrinde* (Barth, Leipzig, 1909).
6. Strick, P. L. & Preston, J. B. *J. Neurophysiol.* **48,** 139–149 (1982).
7. Strick, P. L. & Preston, J. B. *J. Neurophysiol.* **48,** 150–159 (1982).
8. Kawashima, R. *et al. Neuroreport* **6,** 238–240 (1995).
9. Matelli, M., Luppino, G., Fogassi, L. & Rizzolatti, G. *J. Comp. Neurol.* **280,** 468–488 (1989).
10. Stepniewska, I., Preuss, T. M. & Kaas, J. H. *J. Comp. Neurol.* **330,** 238–271 (1993).
11. Stepniewska, I., Preuss, T. M. & Kaas, J. H. *J. Comp. Neurol.* **349,** 558–582 (1994).
12. Stepniewska, I., Preuss, T. M. & Kaas, J. H. *J. Comp. Neurol.* **349,** 536–557 (1994).
13. Schleicher, A. & Zilles, K. *J. Microsc.* **157,** 367–381 (1990).
14. Roland, P. E. & Zilles, K. *Trends Neurosci.* **17,** 458–467 (1994).
15. Schormann, T., von Matthey, M., Dabringhaus, A. & Zilles, K. *Bioimaging* **1,** 119–128 (1993).
16. Schormann, T., Dabringhaus, A. & Zilles, K. *IEEE Trans. Med. Imag.* **14,** 25–35 (1995).
17. Roland, P. E. *et al. Hum. Brain Map.* **1,** 173–184 (1994).
18. Roland, P. E. & Mortensen, E. *Brain Res. Rev.* **12,** 1–42 (1987).
19. Seitz, R. J., Roland, P. E., Bohm, C., Greitz, T. & Stone-Elander, S. *Eur. J. Neurosci.* **3,** 481–492 (1991).
20. Kunesch, E., Binkofski, F. & Freund, H. J. *Exp. Brain Res.* **78,** 539–546 (1989).
21. Merker, B. *J. Neurosci. Methods* **9,** 235–241 (1983).
22. Mahalanobis, P. C., Majumda, D. N. & Rao, C. R. *Sankhya Kalkutta* **9,** 89–324 (1949).
23. Zilles, K. & Schleicher, A. in *Autoradiography and Correlative Imaging* (eds Stumpf, W. & Solomon, H.) 277–307 (Academic, San Diego, 1995).
24. Roland, P. E. & Larsen, B. *Arch. Neurol.* **33,** 551–558 (1976).
25. Roland, P. E., Levin, B., Kawashima, R. & Åkerman, S. *Hum. Brain Map.* **1,** 3–19 (1993).

ACKNOWLEDGEMENTS. We thank K. Rascher for help with the manuscript. This work was supported by grants from the Swedish Medical Research Council, the Deutsche Forschungsgemeinschaft and the BioTech Programme.

CORRESPONDENCE and requests for materials should be addressed to K.Z. (e-mail: zilles@hirn.uni-duesseldorf.de).

Redistribution of synaptic efficacy between neocortical pyramidal neurons

Henry Markram & Misha Tsodyks

Department of Neurobiology, The Weizmann Institute for Science, Rehovot 76100, Israel

EXPERIENCE-dependent potentiation and depression of synaptic strength has been proposed to subserve learning and memory by changing the gain of signals conveyed between neurons[1,2]. Here we examine synaptic plasticity between individual neocortical layer-5 pyramidal neurons. We show that an increase in the synaptic response, induced by pairing action-potential activity in pre- and postsynaptic neurons, was only observed when synaptic input occurred at low frequencies. This frequency-dependent increase in synaptic responses arises because of a redistribution of the available synaptic efficacy and not because of an increase in the efficacy. Redistribution of synaptic efficacy could represent a mechanism to change the content, rather than the gain, of signals conveyed between neurons.

Changes in the amplitude of synaptic responses evoked by single-shock extracellular electrical stimulation of presynaptic fibres are usually considered to reflect a change in the gain of synaptic signals, and are the most frequently used measure for evaluating synaptic plasticity[1–5]. Synapses, however, do not merely transmit each action potential in an identical manner but do so in a manner which is dependent on the history of activity of the synapse[6]. It is therefore not possible to extrapolate from changes in single or even paired synaptic responses to the general behaviour of the synapse. To evaluate changes in the gain of a synapse it is necessary to consider the transmission of synaptic signals more complex than single excitatory postsynaptic potentials (e.p.s.ps) such as those evoked by a train of action potentials. Test synaptic responses evoked by high-frequency trains of extracellular electrical shocks may not only alter the sensitivity of axons to subsequent stimuli, but may also recruit polysynaptic circuits. It is therefore necessary to induce high-frequency transmission

between individual neurons. A preparation was developed[7,8] which enabled high-resolution whole-cell patch-clamp recordings of preselected layer 5 pyramidal neurons (Fig. 1*a*). This preparation enables repeated reliable examination of the same synaptic connection (on average 5.6 synapses per connection; H. Markram, J. Lübke, M. Frotscher, A. Roth and B. Sakmann, manuscript submitted) and has been used to demonstrate the importance of coincident synaptic input and dendritic action potentials in the induction of plasticity at this synaptic connection[8].

A train of presynaptic action potentials at frequencies above about 0.25 Hz results in depression of the synaptic response, until a stationary level of e.p.s.p amplitude is reached (defined here as e.p.s.p_{stat}). This amplitude is reached faster and decreases as the presynaptic action-potential frequency increases. To test the effect of simultaneous burst firing of pre- and postsynaptic neurons (Fig. 1*b*) on subsequent transmission of trains of action potentials, 6–7 e.p.s.ps at 23 Hz were generated by brief current pulses injected into the soma of the presynaptic neuron. An example of how transmission of a high-frequency action potential train changes in this synaptic pathway after pairing pre- and postsynaptic firing, is shown in Fig. 1. During the control period, the synapse seemed to respond randomly to the train (Fig. 1*c*). After pairing, the first action potential in the train almost always generated the largest response (Fig. 1*d*). The increase in this initial e.p.s.p (e.p.s.p_{init}) lasted more than 40–60 min (Fig. 1*e*) consistent with a recent study of these synapses[8]. The effect of pairing was hebbian in the sense that burst firing in only the presynaptic (9 synaptic connections) or in only the postsynaptic (5 synaptic connections) neuron failed to induce comparable changes (Fig. 1*e*). The increase in the single action-potential-evoked e.p.s.p induced by the same pairing protocol is also prevented when *N*-methyl-D-aspartate (NMDA) receptors are blocked at these synapses[8]. On the basis of the changes in e.p.s.p_{init} alone it appears that the gain of the synaptic connection might have increased. The entire synaptic response to the action potential train, however, was not uniformly increased, indicating a more complex effect on transmission.

A further examination of the change induced by pairing revealed that whereas the amplitude of e.p.s.p_{init} was increased in 18 of 19 synaptic connections ($66.5 \pm 17.08\%$; $n = 19$; mean ± s.e.m.; $P < 0.001$) the average amplitude of e.p.s.p_{stat} (see Fig. 2*b*) was unaffected (Fig. 2*a–c*) indicating that the absolute efficacy of the synaptic connection was unaffected. The effect of pairing on the amplitude of the e.p.s.ps generated before the stationary level was reached (defined here as transition e.p.s.ps; see Fig. 2*b*) and consequently the area below the voltage response for the entire train was either increased, decreased or unaffected (no mean change; range $<-50\%$ to $>+50\%$ change). The effect of pairing on e.p.s.p_{init} and the lack of effect on e.p.s.p_{stat} was also observed when extracellular Ca^{2+} concentration was lowered to the physiological level for a rat of this age (1.5 mM (ref. 9); 6 synaptic connections; Fig. 2*c*). The higher apparent failures to evoke an e.p.s.p at lowered extracellular $[Ca^{2+}]$ also enabled detection of a pairing-induced decrease in failure rate (from $21 \pm 5.4\%$ to $5 \pm 1.8\%$) which was not significant at higher $[Ca^{2+}]_o$ because of very few initial apparent failures ($2.6 \pm 2.17\%$

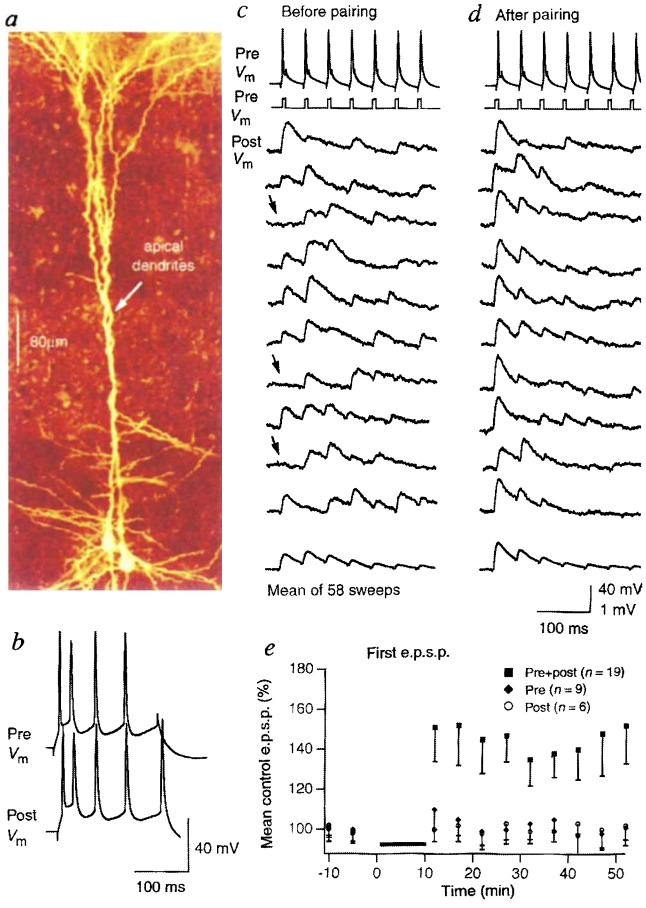

FIG. 1 The effect of pairing pre- and postsynaptic burst firing on high-frequency transmission. *a*, The cell type. A pseudo-coloured collage image of a pair of thick-tufted layer 5 pyramidal neurons loaded with biocytin illustrates the cell type from which all paired recordings were made. *b*, The pairing method. Typically, 200-ms sustained current pulses were co-injected into pre- and postsynaptic neurons with sufficient current to evoke 4–8 spikes. Current pulse in the postsynaptic neurons was delayed (1–5 ms) to ensure that the postsynaptic neuron discharged after onset of synaptic input. No attempt was made to control subsequent spikes. The procedure was repeated 30 times every 20 s. *c*, Test-train responses. A precisely controlled train of presynaptic spikes (23 Hz) (top voltage trace) was generated every 5 s by injecting 2 nA, 5 ms current pulses into the soma of the presynaptic neuron. Responses to action potential trains (postsynaptic membrane voltage (Post V_m) traces). Arrows mark failures of first spike to evoke an e.p.s.p. Control failure rate for the first e.p.s.p. was 12.8% as determined from 58 consecutive sweeps (mean shown in lower trace). *d*, Train response after pairing. The first spike in the train almost always evoked the largest e.p.s.p. Failure rate decreased to 1.7%. *e*, Effect of pairing is lasting and hebbian. Changes in mean amplitude (see Methods) of e.p.s.p_{init} at various times before and after pairing. Separate populations of synaptic connections tested for effects of pairing (Pre + Post), presynaptic burst firing alone (Pre) and postsynaptic burst firing alone (Post). Bar indicates the pairing period.
METHODS. Sagittal slices (300 µM) were cut from the neocortex of Wistar rats (13–15 d) as described in H. Markram *et al.* (manuscript submitted). All experiments were done at 30–32 °C within 6.5 h after slicing. The extracellular solution contained (in mM); 125 NaCl, 2.5 KCl, 25 glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$ and 1 $MgCl_2$. Layer 5 pyramidal neurons from the somatosensory cortical area were identified using infrared differential interference contrast video-microscopy on an upright microscope (Zeiss-Axioskop-FS, fitted with ×40-W/0.75NA objective) as described[16]. Somatic whole-cell recordings (10–20 MΩ access resistance) signals were amplified using two Axoclamp-2B amplifiers (Axon Instruments) and captured on computer using pulse control (by R. Bookman and co-workers, Miami University) and analysed using *IGOR* (Igor Wavemetrics, Lake Oswego, OR). Recordings were made using pipettes containing (in mM); 100K-gluconate, 20 KCl, 4 ATP–Mg, 10 phosphocreatine, 0.03 GTP, 10 HEPES buffer and 0.5% biocytin (pH 7.3, 310 mOsm). Resting membrane-potential levels typically -62 ± 2 mV. Independent and paired *t*-tests were used to determine significance of changes. All data are presented as mean ± s.e.m. Data after pairing or after control protocol represent maximal changes (positive or negative) recorded over a 5-min period (20–60 sweeps) 10–40 min after the end of the pairing period. Whole-cell recordings from two cells were made virtually simultaneously after the configuration was established on both neurons. Pairing was initiated 10–15 min after whole-cell recording. Input and access resistances were monitored by producing brief hyperpolarizing or depolarizing current pulses in the somata after every synaptic response. Responses to presynaptic spike trains were recorded every 15 s in 55 of 59 synaptic connections examined and every 5 s in the remaining 4. Eight synaptic connections were discarded because of unreliable mean sweeps caused by excessively high variation or unstable membrane potentials.

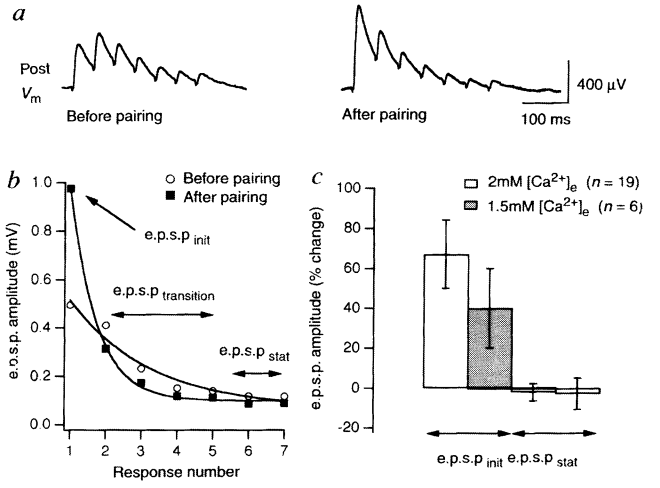

FIG. 2 Pairing induces an increase in initial e.p.s.ps and has no effect on stationary e.p.s.ps. *a*, The mean of 58 sweeps during the control period (~5 min; left trace) and maximally changed mean of 59 sweeps recorded 20 min after pairing. *b*, e.p.s.p. amplitudes in *a* were measured from the voltage immediately before the onset of the e.p.s.p. to the peak of the e.p.s.p. Superimposed is a single exponential fit. Defined are initial, transition and stationary e.p.s.p.s. The amplitude of e.p.s.p_{stat} was taken as the average of the last 50 points of the fitted curve which was equivalent to the last 20% of the curve and roughly equivalent to the average of the last 2 e.p.s.ps. This approach was adopted because the coefficient of variation of later e.p.s.ps is typically above 100% (compared to around 40% for e.p.s.p_{init}) and the amplitudes of individual e.p.s.ps are therefore less reliable given the restricted number of sweeps. The accuracy of this approach to reflect the amplitude of e.p.s.p_{stat} was empirically confirmed by averaging progressively larger number of sweeps and comparing the amplitudes. *c*, e.p.s.p_{init} and e.p.s.p_{stat} in two separate synaptic populations in which $[Ca^{2+}]_o$ was either 2 or 1.5 mM. In 2 mM $[Ca^{2+}]_o$ pre-pairing mean of e.p.s.p_{init} is 1.17 ± 0.229 mV and e.p.s.p_{stat} is 184 ± 28 μV; $n = 19$.

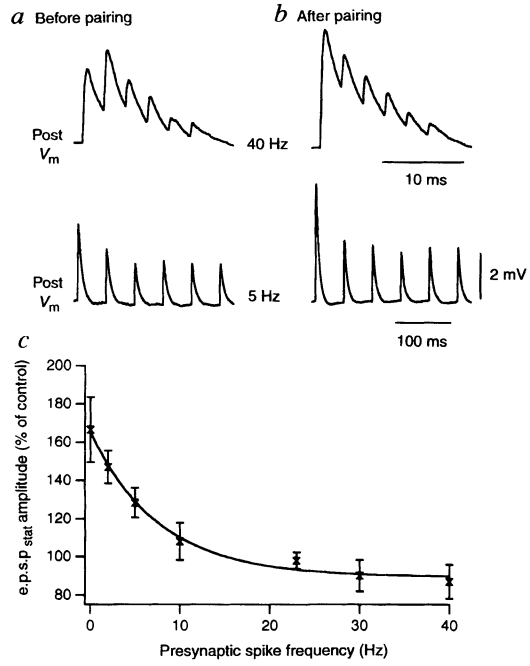

FIG. 3 Frequency dependence of synaptic potentiation. *a*, *b*, Results from an experiment on a single synaptic connection in which e.p.s.ps were generated alternately by 5-Hz and 40-Hz presynaptic spike trains before and after pairing. e.p.s.p_{init} is increased to the same extent for both frequencies, e.p.s.p_{stat} is not changed in the 40-Hz trace but is increased in the 5-Hz trace (compare for example the amplitude of the last 2 e.p.s.ps in the 40-Hz train before and after pairing). Even at 5 Hz, the e.p.s.p_{stat} was only increased by 25% whereas e.p.s.p_{init} was increased by 58%. *c*, Summary of the change in e.p.s.p_{stat}. Total of 33 synaptic connections represented (1–4 frequencies tested per synaptic connection). The first, leftmost point represents very-low-frequency presynaptic spikes (0.25 Hz in 2 synaptic connections and 0.067 Hz in 17 synaptic connections) and actually are the amplitudes of e.p.s.p_{init} of Fig. 2. Second point, 2 Hz ($n = 11$). Third point, 5 Hz ($n = 10$). Fourth point, 23 Hz ($n = 19$). Fifth point 30 Hz ($n = 4$) and last point, 40 Hz ($n = 9$).

before pairing and 0.33 ± 0.25% after pairing).

A more rapid depression of synaptic responses during the spike train is characteristic of the change induced by pairing (see Fig. 2*b*). The time constant for the decay of the amplitudes (defined as DTC) of successive e.p.s.ps decreased by 30% from (52 ± 6.7 ms to 36 ± 4.3 ms) whereas the time constant for e.p.s.p_{init} to recover to its maximum was unaffected by pairing (1.135 ± 0.034 ms before and 1.133 ± 0.051 ms after; 11 synaptic connections). The DTC value before pairing indicated the status of the synaptic connection and could therefore be used to predict quite accurately the change in e.p.s.p_{init} and in the DTC after pairing (positive correlation between pre-pairing DTC and change in e.p.s.p_{init}; $r = 0.92$). The natural range of DTC values observed before pairing is most likely due to differences in the use of the available efficacy by each synaptic connection (leaving more or less efficacy for subsequent action potentials) which could result from a continuum of release probabilities within a synaptic population[10].

The increase in the rate of synaptic depression after pairing was not caused by indirect effects on polysynaptic transmission as synaptic depression was not affected by the blockade of γ-amino butyric acid (GABA) receptors with picrotoxin (5 μM, Sigma) and saclophen (10 μM; Tocris) (3 synaptic connections; not shown) or by blockade of metabotropic glutamate receptors with 2-amino-5-phosphonopropionic acid (10 μM; Tocris; 2 synaptic connections; not shown). Synaptic depression was also not affected when postsynaptic voltage-activated channels were blocked after re-patching and loading neurons with the lidocaine derivative, *N*-ethylbromide quaternary salt (QX-314, 5 μM, Research Biochemical Inc., 5 synaptic connections, not shown).

The increase in the amplitude of e.p.s.p_{init}, which actually represents e.p.s.p_{stat} for very low-frequency stimulation (<0.25 Hz), and the lack of an effect on the amplitude of e.p.s.p_{stat} for a high-frequency train, indicates that the potentiation is conditional on the presynaptic spike frequency. The effect of pairing on e.p.s.p_{stat} at several different frequencies was therefore examined. In the same neuron, e.p.s.p_{init} was found to increase equally for low- and high-frequency trains, whereas e.p.s.p_{stat} was increased only when the frequency was low (Fig. 3*a*, *b*). Potentiation of synaptic responses therefore only occurred when the presynaptic frequency was below 20 Hz (Fig. 3*c*).

The lack of an effect on the e.p.s.p_{stat} level indicates that postsynaptic receptors were not recruited or potentiated[11–13] nor were silent synapses unmasked[14]. As pairing did not affect the rate of recovery from depression, it would seem that the actual biophysical mechanism of synaptic depression was also unaffected by pairing and that the increased rate of depression was secondary to increased use of the available efficacy of the synaptic connection by action potentials. The most likely mechanism for increased use of the existing synaptic efficacy is an increase in the probability of transmitter release after pairing (see also ref. 15), but it could also be generated by an increase in the affinity of postsynaptic receptors for glutamate if these receptors are not saturated.

The effect reported here is not an unconditional potentiation of the efficacy of the synaptic connection; instead, it is a redistribution of the existing efficacy between spikes in a train. This type of plasticity should be distinguished from unqualified potentiation or depression of synaptic efficacy, which imply a change in the gain of synaptic signals transmitted and which would be produced by

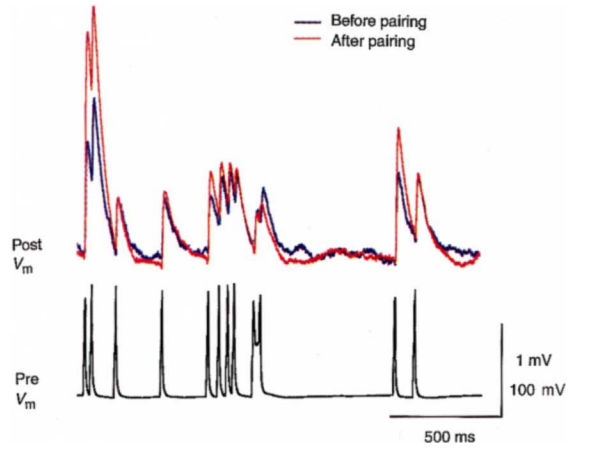

FIG. 4 Complex effect of pairing on the synaptic response generated by an irregular presynaptic action potential train. An irregular spike train was generated by brief (5 ms) current pulses repeatedly for 30 episodes and the postsynaptic response averaged before (blue) and after (red) pairing.

mechanisms such as postsynaptic changes or by adding or removing synapses to or from the connection. Redistribution of synaptic efficacy could potentially occur at any synapse in the brain if activated at a faster rate than required for complete recovery of the synaptic efficacy.

The physiological implications of redistribution of efficacy are also entirely different from unconditional potentiation or depression and are partly predicted by the frequency-dependent potentiation seen in Fig. 3. Under *in vivo* conditions, neurons tend to discharge irregularly, which effectively represents a multitude of spike frequencies persisting for different time periods, indicating that the effect of pairing on synaptic input generated by an irregular presynaptic spike train would be complex if redistribution of synaptic efficacy was to occur (Fig. 4). The effect cannot be predicted as most synaptic responses during such a train have not reached a stationary level for the given frequency and hence are all transition e.p.s.ps (see Fig. 2*b*). As discussed, transition e.p.s.ps could be enhanced, depressed or unchanged after pairing. Redistribution of synaptic efficacy may therefore serve as a powerful mechanism to alter the dynamics of synaptic transmission in subtle ways and hence to alter the content rather than the gain of signals conveyed between neurons. □

Received 21 February; accepted 28 June 1996.

1. Teyler, T. J. & Discenna, P. *Brain Res.* **319,** 15–28 (1984).
2. Barnes, C. A. *Neuron* **15,** 751–754 (1995).
3. Stevens, C. F. *Neuron* **10,** 55–63 (1993).
4. Kullman, D. M. & Siegelbaum, S. A. *Neuron* **15,** 997–1002 (1995).
5. Bliss, T. V. P. & Lomo, T. *J. Physiol. (Lond.)* **232,** 331–336 (1973).
6. Thomson, A. M. & Deuchars, J. *Trends Neurosci.* **17,** 119–126 (1994).
7. Markram, H., Lübke, J., Sakmann, B. & Frotscher, M. in *European Neuroscience Association Abstr.* **34.27** (1995).
8. Markram, H. & Sakmann, B. in *Soc. Neurosci. Abstr.* 788.11 (1995).
9. Jones, H. C. & Keep, R. F. *J. Physiol. (Lond.)* **P64** (1987).
10. Allen, C. & Stevens, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **91,** 10380–10383 (1994).
11. McNaughton, B. L. *J. Physiol. (Lond.)* **324,** 249–262 (1982).
12. Kauer, J. A., Malenka, R. C. & Nicoll, R. A. *Neuron* **1,** 911–917 (1988).
13. Bashir, Z. I., Alford, S., Davies, S. N., Randall, A. D. & Collingridge, G. L. *Nature* **349,** 156–158 (1991).
14. Liao, D. Z., Hessler, N. A. & Malinow, R. *Nature* **375,** 400–404 (1995).
15. Stevens, C. F. & Wang, Y. *Nature* **371,** 704–707 (1994).
16. Stuart, G. J., Dodt, H.-U. & Sakmann, B. *Pflügers Archiv.* **423,** 511–518 (1992).

ACKNOWLEDGEMENTS. We thank G. Borst for helpful comments on the study; M. Segal, E. Ahissar, A. Aertsen, Y. Dudai, R. Rahamimoff, W. Singer, B. Katz, R. Douglas, and I. Segev for their comments on the manuscript; D. Pikus for programming and data analysis and A. Gupta for histology. H.M. holds the Joseph D. Shane career-development chair. The study was supported by research grants from the Henry S. and Ann S. Reich Research Fund for Mental Health, the Minerva Foundation and the ONR.

CORRESPONDENCE and requests for materials should be addressed to H.M. (e-mail: bnmark@weizmann.weizmann.ac.il).

A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms

Rae Silver, Joseph LeSauter, Patrick A. Tresco* & Michael N. Lehman†

Departments of Psychology, Barnard College and Columbia Univeristy, 3009 Broadway, New York, New York 10027, USA
* Department of Bioengineering, Room 506 BPR, University of Utah, Salt Lake City, Utah 84112, USA
† Department of Cell Biology, Neurobiology and Anatomy, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267, USA

THE mammalian suprachiasmatic nuclei (SCN) transmit signals to the rest of the brain, organizing circadian rhythms throughout the body[1–4]. Transplants of the SCN restore circadian activity rhythms to animals whose own SCN have been ablated[5–9]. The nature of the coupling signal from the grafted SCN to the host brain is not known, although it has been presumed that functional recovery requires re-establishment of appropriate synaptic connections. We have isolated SCN tissue from hamsters within a semipermeable polymeric capsule before transplantation, thereby preventing neural outgrowth but allowing diffusion of humoral signals. Here we show that the transplanted SCN, like neural pacemakers of *Drosophila*[10] and silkmoths[11], can sustain circadian activity rhythms by means of a diffusible signal.

To show that the recovered circadian rhythm is produced by the donor tissue and is not a consequence of spontaneous recovery of function, the host animals used were male hamsters bearing the *tau* mutation[12] and were raised in our colony. This mutation has the behavioural effect of altering the period of the endogenous circadian rhythm to about 22 hours in heterozygotes and 20 hours in homozygotes. Donor animals were fetuses (embryonic day 14–15) from wild-type, time-pregnant females (Charles River, MA). Because of the difference in period length between host and donor phenotype, the period of the locomotor rhythm could be unambiguously attributed to either the donor SCN or the host animal[13].

Animals were housed individually in cages equipped with running wheels, and were allowed unlimited access to food and water. The cages were placed in a room in constant darkness (DD) equipped with a dim red light (<1 lux; Delta 1, Dallas) to allow for animal maintenance and a white-noise generator (91 dB) to mask environmental noise, and maintained at about 23 °C. After the host rhythm had been characterized (for 7–14 days), hamsters were anaesthetized with pentobarbital (100 mg per kg) and bilateral lesions of the SCN were made as previously described[9]. Animals in which 24-hour periodicity was not seen upon inspection of the actogram were used as hosts for implantation (see Fig. 1 legend). The extent of the lesion was determined histologically at the end of the experiment.

After behavioural testing, hamsters were heavily anaesthetized (pentobarbital 200 mg per kg) and perfused intracardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3. Coronal sections (20 μm) were cut on a cryostat and alternate sections were immunostained for vasoactive intestinal polypeptide (VIP), vasopressin-associated neurophysin (NP), cholecystokinin (CCK), or calbindin-D28K protein (CaBP), to verify the lesion and the presence of neurochemicals characteristic of the SCN in the encapsulated graft. Polyclonal antisera to VIP, NP, CCK (Incstar, MN) and CaBP (Sigma, MO) were obtained and these antigens were detected using a modified avidin-biotin-HRP procedure (Vectastain Elite Kit, Vector Laboratories, CA).