33. M. Frankignoulle *et al.*, *Science* **282**, 434 (1998).
34. R. A. Houghton, J. L. Hackler, K. T. Lawrence, *Science* **285**, 574 (1999).
35. S. Pacala *et al.*, *Science* **292**, 2316 (2001).
36. S. V. Smith, W. H. Renwick, R. W. Buddemeier, C. J. Crossland, *Global Biogeochem. Cyc.* **15**, 697 (2001).
37. R. W. Howarth, J. R. Fruci, D. Sherman, *Ecol. Appl.* **1**, 27 (1991).
38. J. A. Lynch, J. W. Grimm, V. C. Bowersox, *Atmos. Environ.* **29**, 1231 (1995)
39. R. Nemani *et al.*, *Geophys. Res. Lett.* **29**, 106 (2002).
40. J. A. Hicke *et al.*, *Geophys. Res. Lett.* **29**, 69 (2002).
41. R. E. Turner, N. N. Rabalais, *Nature* **368**, 619 (1994).
42. D. A. Goolsby, W. A. Battaglin, *Hydrol. Proc.* **15**, 1209 (2001).
43. W. Schlesinger, J. A. Andrews, *Biogeochemistry* **48**, 7 (2000).
44. U. Skiba, K. J. Hargreaves, D. Fowler, K. A. Smith, *Atmos. Environ.* **26**, 2477 (1992).
45. K. Semhi, P. Amiotte-Suchet, N. Clauer, J.-L. Probst, *Appl. Geochem.* **15**, 865 (2000).
46. We thank D. Flowers of the USGS, who was extremely helpful in helping us compile all discharge data presented in this paper; J. Milliman for his helpful review and advice; and K. Turekian for numerous conversations on the subject. Financial support was provided in part by NSF. This paper is a contribution to the Yale School of Forestry and Environmental Studies and the Institute of Ecosystem Studies.

Supporting Online Material
www.sciencemag.org/cgi/content/full/301/5629/88/DC1
Figs. S1 and S2

24 February 2003; accepted 15 May 2003

Pharmacological Modulation of Perceptual Learning and Associated Cortical Reorganization

Hubert R. Dinse,[1]* Patrick Ragert,[1] Burkhard Pleger,[2] Peter Schwenkreis,[2] Martin Tegenthoff[2]

The pharmacological basis of perceptual learning and associated cortical reorganizations remains elusive. We induced perceptual learning by Hebbian coactivation of the skin of the tip of the right index finger in humans. Under placebo, tactile two-point discrimination was improved on the coactivated but not on the left index finger. This augmentation was blocked by an *N*-methyl-D-aspartate–receptor blocker, but doubled by amphetamine. No drug effects were found on the left index finger. The individual amount of cortical reorganization as assessed by mapping of somatosensory evoked potentials was linearly correlated with the pharmacological modulation of discrimination thresholds, implying that perceptual learning and associated cortical changes are controlled by basic mechanisms known to mediate and modulate synaptic plasticity.

Cellular studies suggest that there might be only a few basic mechanisms that control synaptic transmission. In particular, the *N*-methyl-D-aspartate (NMDA) receptor has been implicated in synaptic plasticity (*1*, *2*). Alterations of synaptic efficacy can be modulated by other pharmacological agents, thereby acting to gate synaptic plasticity (*3*–*5*). However, at a behavioral level, the pharmacological mechanisms mediating perceptual learning and associated cortical reorganization in humans remain to be clarified.

To study a particular form of perceptual learning and associated cortical changes, we recently introduced a coactivation protocol that closely follows the idea of Hebbian learning (fig. S1): Synchronous neural activity, necessary to drive plastic changes, is evoked by coactivation for a few hours of small skin portions of the tip of the right index finger (IF) (*6*–*9*). As a result, the finger representation in the somatosensory cortex enlarges and tactile discrimination performance improves (*6*–*9*).

Here we used the coactivation protocol to study the pharmacological mechanisms that underlie perceptual learning by combining psychophysics and mapping of somatosensory evoked potentials (SSEPs) in human subjects. To scrutinize the apparently ubiquitous role of NMDA receptors, we applied memantine, which blocks NMDA receptors (*10*). Although there are many approaches to block plastic processes, less is known about drugs that enhance cortical plasticity. We therefore applied amphetamine in a single dose (*11*) to test its modulatory role in learning processes evoked by the coactivation protocol.

In placebo-controlled human subjects, 3 hours of tactile coactivation on the tip of the IF lowered discrimination thresholds for the spatial two-point discrimination task (Fig. 1) [analysis of variance (ANOVA), $F = 8.887$, $P = 0.009$, pre-post difference post hoc $P < 0.005$, $n = 16$ subjects]. After coactivation, psychometric functions showed a distinct shift of thresholds toward smaller separation distances (Fig. 2). Assessment 24 and 48 hours after coactivation revealed normal pre-coactivation thresholds, confirming the reversibility of changes. We found no significant correlation between individual pre-coactivation thresholds and the amount of improvement (Pearson's $r = -0.173$, $P > 0.5$, $n = 16$ subjects). As a control, and to demonstrate the specificity of the coactivation-induced changes, we measured thresholds of the IF of the left hand, which was not coactivated. Thresholds remained unchanged ($P = 0.234$) (Figs. 1 and 2), confirming the lack of generalization of coactivation across hands (*6*–*9*).

Memantine completely eliminated the coactivation-induced gain in discrimination

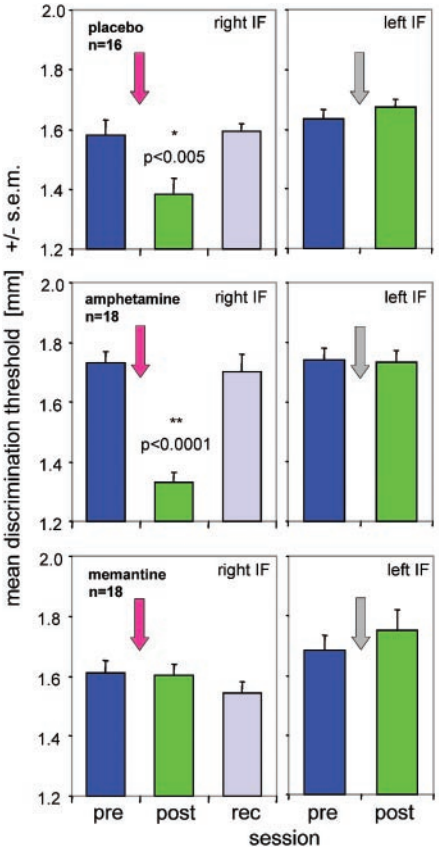

Fig. 1. The pharmacological modulation of coactivation effects on discrimination thresholds (mean ± SEM). The 3-hour coactivation episode applied to the tip of the right IF is indicated by pink arrows for the right hand and gray arrows for the left hand. For each group, discrimination thresholds obtained for the test finger (right IF) are shown pre- and post-coactivation and 24 hours after coactivation (rec). For the control finger (left IF, which was not coactivated), thresholds are shown for the pre- and post-coactivation conditions. The general lack of effects for the control finger indicates the finger-specificity of the coactivation protocol (in the placebo group) and a lack of unspecific side effects (in the drug groups). *, $P < 0.005$; **, $P < 0.0001$.

[1]Institute for Neuroinformatics, Department of Theoretical Biology, Ruhr-University Bochum, D-44780 Bochum, Germany. [2]Department of Neurology, Ruhr-University Bochum, BG-Kliniken Bergmannsheil, D-44789 Bochum, Germany.

*To whom correspondence should be addressed. E-mail: hubert.dinse@neuroinformatik.ruhr-uni-bochum.de

abilities (Figs. 1 and 2) (ANOVA, $F = 0.277$, $P = 0.924$, pre-post difference post hoc $P = 0.773$, $n = 18$ subjects). To rule out memantine interference with normal discrimination, we assessed the thresholds of the left IF before and after memantine administration. Thresholds were not significantly affected (*t* test, $P = 0.43$) (Fig. 1), indicating that blocking of NMDA receptors had no unspecific effects on thresholds.

In contrast, a single dose of amphetamine boosted the coactivation-induced improvement beyond previously observed levels (ANOVA, $F = 35.674$, $P < 0.0001$, pre-post difference post hoc $P < 0.0001$, $n = 18$ subjects) (Figs. 1 and 2). Although in placebo-controlled subjects coactivation lowered thresholds by 0.20 mm (a 12.6% gain in performance), coactivation under amphetamine led to a 0.4-mm lowering (a 23.1% gain, $P < 0.005$). Given the broad spectrum of amphetamine effects, it was important to show that the drug did not affect spatial discrimination per se, which was confirmed by the lack of effects on the left IF (*t* test, $P = 0.68$, $n = 18$ subjects).

To study the pharmacological modulation of coactivation effects on the digit representation in the primary somatosensory cortex, we mapped the SSEPs and calculated the N20-dipole locations after electrical stimulation of the IF of each hand in 24 subjects. Thresholds of these subjects were in the same range as those of the population as a whole. Results of the SSEP analysis are summarized in Table 1. For the IF of the right hand that underwent coactivation, we found that, in the placebo group, the Euclidean distance between the dipole pre- and post-coactivation was significantly larger on the coactivated side than on the control side ($P = 0.01$, $n = 8$ subjects). In the left hemisphere, the polar angle of the N20-dipole locations of the coactivated IF increased after coactivation ($P = 0.001$), but no changes were found in the right hemisphere for the left IF ($P = 0.45$). These results indicate a lateral and inferior shift on the postcentral gyrus of the left hemisphere that represents the coactivated index finger (Fig. 3). Dipole strength increased on the coactivated hemisphere ($P = 0.04$), but not on the contralateral side ($P = 0.96$). The goodness of fit (GOF) was not affected.

In the memantine group, the lack of coactivation-induced improvement of discrimination was paralleled by a lack of changes in the SSEPs. The Euclidean distance between the dipole pre- and post-coactivation on the coactivated side was not different from the distance on the control side ($P = 0.97$, $n = 8$ subjects), and no changes were observed for the polar angle of the coactivated IF ($P = 0.27$). Similarly, dipole strength ($P = 0.22$) and GOF ($P = 0.22$) remained unaffected. The lack of unspecific side effects of memantine on discrimination of the left IF was confirmed by an absence of effects in the right hemisphere for all SSEP parameters.

Amphetamine application increased the Euclidean distance between the dipole pre- and post-coactivation on the coactivated side as compared to the control side ($P < 0.001$, $n = 8$ subjects). The polar angle of the N20 dipole of the coactivated IF also increased ($P < 0.001$), as did dipole strength ($P = 0.03$), whereas GOF remained unaffected. In the right hemisphere, no amphetamine-induced changes for the N20 dipole for the left IF could be found.

The results obtained in the placebo and the amphetamine groups indicate a substantial lateral and inferior shift of the N20 dipole on the postcentral gyrus of the left hemisphere that represents the coactivated index finger. In contrast, no significant changes were found on the contralateral hemisphere, or on either hemisphere in the memantine group (Fig. 3 and Table 1). However, for the amphetamine group, the coactivation- induced lateral shift was even larger than in the placebo group ($P < 0.01$).

The pharmacologically induced effects on tactile discrimination all varied individually,

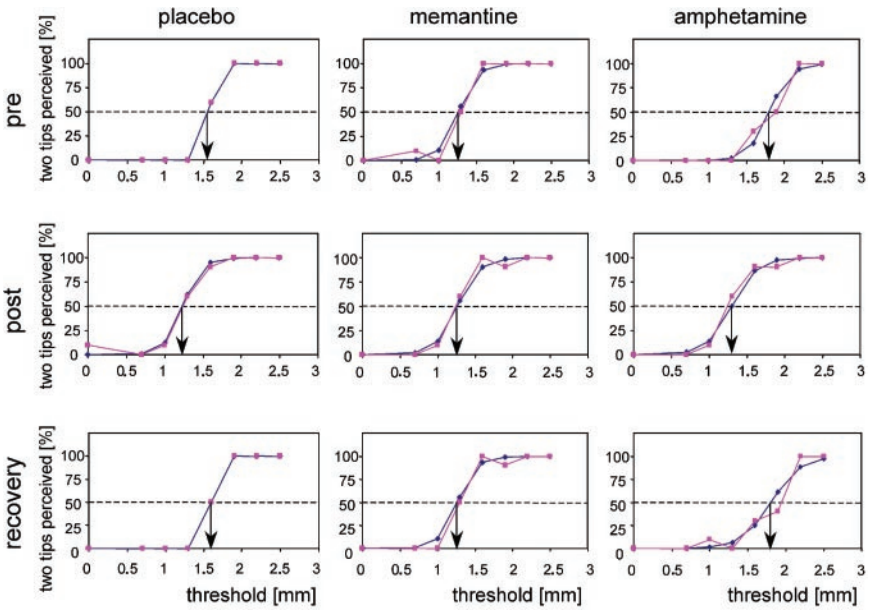

Fig. 2. Psychometric functions illustrating the coactivation-induced effects on discrimination threshold for an individual subject from each group (placebo, memantine, and amphetamine). Correct responses in percent (pink symbols) are plotted as a function of the separation distance together with the results of a logistic regression (blue line). Top row: pre-coactivation; middle row: immediately after coactivation; bottom row: recovery after 24 hours. The 50% level of correct responses is indicated (dashed line) together with the resulting thresholds (arrows).

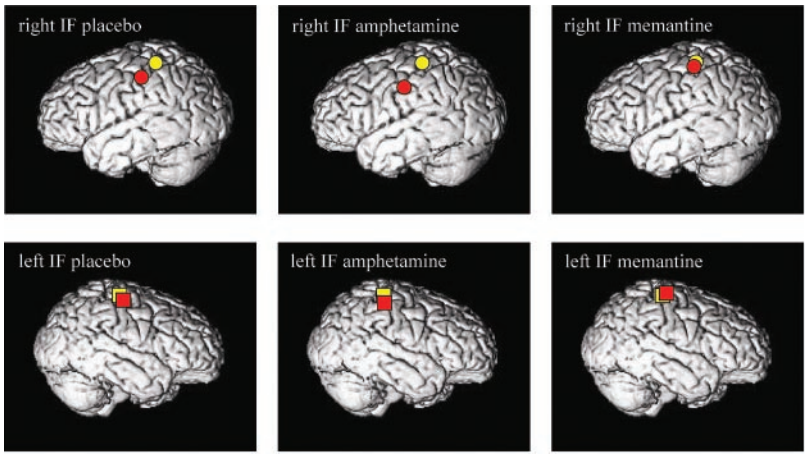

Fig. 3. A schematic projection of the average locations of the single-equivalent N20 dipoles of the IF pre- (yellow symbols) and post- (red symbols) coactivation onto three-dimensional reconstructed magnetic resonance images of an individual subject. The coactivation-induced shift toward the lateral and inferior aspects of the postcentral gyrus in the placebo-controlled group is nearly doubled in the amphetamine group, but blocked under memantine. Comparable effects are lacking on the not-coactivated hemisphere (bottom row). Circles, dipole locations on the coactivated hemisphere; squares, dipole locations on the non-coactivated hemisphere.

as did the dipole localizations observed. Under the assumption that SSEP changes reflect changes in cortical processing that are causally related to the processing of information relevant to the discrimination task, we hypothesized that the observed dipole changes should correlate with changes in individual performance. A linear correlation analysis revealed a significant relationship between the coactivation-induced dipole changes and parallel changes in discrimination thresholds (Fig. 4). This was true for Euclidean distance (left-right normalized, Pearson's $r = 0.640$, $P = 0.001$, $n = 24$ subjects), polar angles (Pearson's $r = 0.736$, $P < 0.001$) (Fig. 4, left), and mediolateral dipole shifts (Pearson's $r = 0.824$, $P < 0.0001$) (Fig. 4, right). The correlation analysis preserved a distinct clustering according to pharmacological treatment: Little gain in spatial discrimination abilities was associated with small changes in dipole shifts. On the other hand, those subjects who showed a large cortical reorganization also had low thresholds. Subjects who failed to show any perceptual improvement because of the application of memantine showed no or only very weak changes in dipole localization. The highest gain in performance characteristic for amphetamine-treated subjects was also paralleled by the largest reorganization observed (Fig. 4).

Coactivation is a task-free, passive stimulation protocol. Many studies have demonstrated that plastic changes can be evoked by the variation of input statistics alone, without invocation of attention or reinforcement, provided the statistics are sufficiently altered (*12–14*). Perceptual learning occurs even without awareness of stimuli, through repetitive exposure to stimuli that are below threshold (*15*). Our results provide further evidence that perceptual performance can be improved solely by manipulation of the input statistics. We also demonstrate that the perceptual improvement induced by tactile coactivation is controlled by pharmacological agents that modulate synaptic plasticity. These modulations did not result in unspecific excitability changes, but the drug-induced changes in perceptual thresholds were linearly correlated with corresponding changes in SSEP dipole localization in the somatosensory cortex. Accordingly, small differences in performance may not be necessarily due to measurement artifacts or noise, but may reflect true differences in individual brain organization.

The coactivation-induced improvement in discrimination performance was highly specific with no transfer to the non-coactivated hand (Fig. 1) (*6–9*). We therefore used the performance of the left, non-coactivated IF to demonstrate the specificity of the observed drug effects. All substances had no effect per se on spatial discrimination performance (Fig. 1), which, together with the consistency of the effects across subjects, supports the specific nature of the drug effects.

Our results provide evidence that NMDA-receptor activation is required for the manifestation of this particular type of fast, coactivation-induced perceptual learning, which is consistent with the involvement of mechanisms mediated by γ-aminobutyric acid as reported recently (*16*). In the human motor system, practicing movements has been shown to improve performance (*17*, *18*). These training effects can be

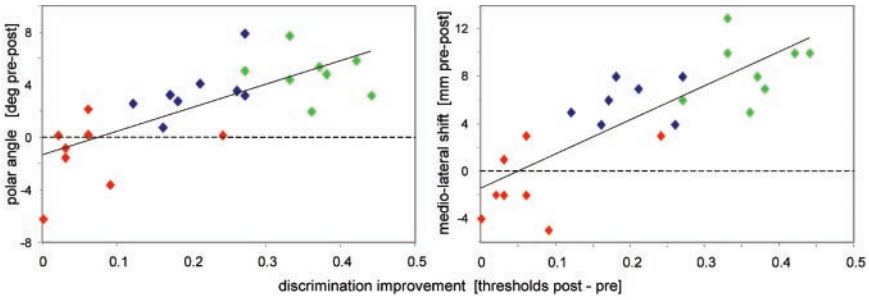

Fig. 4. Correlation between coactivation-induced changes of (**left**) the polar angles (degree pre-post) and the changes of two-point discrimination thresholds for individual subjects, and (**right**) between mediolateral dipole shifts and threshold changes. The correlation analysis preserves a distinct clustering according to the pharmacological treatment. Red, memantine; blue, placebo; green, amphetamine.

Table 1. Pharmacological effects on SSEP parameters (mean ± SEM). Pre- and post-coactivation results are shown ($n = 8$ subjects for each group).

SSEP parameter	Placebo		Memantine		Amphetamine	
			Euclidean distance (mm pre-post)			
Right IF – left hemisphere	9.03 ± 1.13†		6.20 ± 1.48		13.31 ± 0.91‡§	
Left IF – right hemisphere	5.42 ± 0.96		6.25 ± 0.88		5.38 ± 0.79	
	pre	post	pre	post	pre	post
			Polar angle (degrees)			
Right IF – left hemisphere	23.81 ± 1.51	27.42 ± 1.83*	31.77 ± 2.48	30.67 ± 1.82	24.23 ± 1.46	29.11 ± 1.89***
Left IF – right hemisphere	31.00 ± 6.55	30.18 ± 5.85	35.19 ± 6.47	34.32 ± 5.98	28.88 ± 5.56	27.92 ± 5.80
			Dipole strength (nA)			
Right IF – left hemisphere	2.68 ± 0.43	3.67 ± 0.33*	2.90 ± 0.64	2.22 ± 0.50	2.65 ± 0.37	3.60 ± 0.27**
Left IF – right hemisphere	4.40 ± 0.90	4.01 ± 0.70	2.73 ± 0.45	2.65 ± 1.92	3.70 ± 0.54	4.18 ± 0.68
			GOF (%)			
Right IF – left hemisphere	96.3 ± 0.35	96.8 ± 0.44	97.00 ± 0.71	96.00 ± 0.53	94.00 ± 2.12	95.00 ± 0.99
Left IF – right hemisphere	96.7 ± 0.47	97.3 ± 0.71	95.00 ± 0.45	96.00 ± 0.31	96.00 ± 0.46	96.00 ± 0.74

Significantly different from the pre-coactivation condition: *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$. †Significantly different from the control (right hemisphere): $P = 0.01$. ‡Significantly different from the control (right hemisphere): $P < 0.001$. §Left-right difference significantly different from the placebo condition: $P < 0.01$.

reduced by the application of NMDA-receptor blockers, indicating that NMDA-receptor activation operates in use-dependent plasticity in the human motor cortex (*17*, *18*).

As a chain of changes, we suggest that the simultaneous activation of the skin modifies synaptic efficacy between and within the cortical neuron pool that represents the IF (*19*), which results in an enlargement of the finger representation in SI (*8*, *9*) and SII (*9*). Single-cell recordings in rat SI after coactivation revealed an enlargement of the cortical representations of the coactivated skin sites (*6*) and persistent long-term potentiation (LTP)–like changes in responsiveness (*20*), which is compatible with the described NMDA dependence of coactivation.

The modulatory role of amphetamine might be related to the enhancing effects of amphetamine on LTP (*4*). Amphetamine, when administered peripherally, increases centrally the levels of dopamine, serotonin, and noradrenaline. Monoamines modify long-term changes in synaptic function, with 5-hydroxytryptamine (serotonin) being more potent than noradrenaline (*3*, *5*). Modifying effects can also be exerted by the widespread neuromodulatory projections received by the neocortex (*21*, *22*). Ventral tegmental neurons are believed to provide reinforcement signals for learning-related reorganization (*23*). Possibly, amphetamine-induced release of dopamine might amplify coactivation-induced learning processes in a manner similar to that reported after ventral tegmental stimulation is paired with auditory stimuli (*24*), but lead to no change when no consistent activation is provided. Recently, the role of amphetamine in promoting the development of use-dependent plasticity in the human motor cortex has been reported (*25*). Using a single dose of amphetamine, we demonstrate that the effect of coactivation was boosted both perceptually and neurophysiologically, providing further evidence that perceptual learning is subject to amplification by amphetamine.

Human N20 dipoles obtained for finger stimulation have been shown to be localized in area 3b of the primary somatosensory area (*26*, *27*). Our results provide further evidence that changes in early cortical areas might be more directly linked to perceptual learning than previously thought. The high effectiveness of memantine argues for a broad and rather ubiquitous involvement of NMDA receptors, whereas the potentiating effects of amphetamine support the role of adrenergic substances in the modulation of perceptual learning.

References and Notes

1. T. V. Bliss, G. L. Collingridge, *Nature* **361**, 31 (1993).
2. R. A. Nicoll, R. C. Malenka, *Nature* **377**, 115 (1995).
3. T. V. Bliss, G. V. Goddard, M. Riives, *J. Physiol.* **334**, 475 (1983).
4. R. L. Delanoy, D. L. Tucci, P. E. Gold, *Pharmacol. Biochem. Behav.* **18**, 137 (1983).
5. J. C. Lacaille, C. W. Harley, *Brain Res.* **358**, 210 (1985).
6. B. Godde, F. Spengler, H. R. Dinse, *Neuroreport* **8,** 281 (1996).
7. B. Godde, B. Stauffenberg, F. Spengler, H. R. Dinse, *J. Neurosci.* **20,** 1597 (2000).
8. B. Pleger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 12255 (2001).
9. B. Pleger *et al.*, *Soc. Neurosci. Abstr.* **28**, 841.7 (2002).
10. C. G. Parsons, W. Danysz, G. Quack, *Neuropharmacology* **38**, 735 (1999).
11. B. Angrist, J. Corwin, B. Bartlik, T. Cooper, *Biol. Psychiatry* **22**, 1357 (1987).
12. M. E. Diamond, M. Armstrong-James, F. F. Ebner, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2082 (1993).
13. M. P. Kilgard, P. K. Pandya, N. D. Engineer, R. Moucha, *Biol. Cybern.* **87**, 333 (2002).
14. H. R. Dinse, M. M. Merzenich, in *Perceptual Learning*, M. Fahle, T. Poggio, Eds. (MIT Press, Boston, 2002), pp. 19–42.
15. T. Watanabe, J. E. Nanez, Y. Sasaki, *Nature* **413**, 844 (2001).
16. H. R. Dinse, P. Ragert, B. Pleger, P. Schwenkreis, M. Tegenthoff, *Neuroreport*, in press.
17. M. Tegenthoff, K. Witscher, P. Schwenkreis, J. Liepert, *Electroencephalogr. Clin. Neurophysiol. Suppl.* **51**, 188 (1999).
18. C. M. Bütefisch *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3661 (2000).
19. Dendritic spikes mediate a form of synaptic potentiation that does not require postsynaptic action-potential firing in the axon (*28*).
20. H. R. Dinse, K. Kreikemeier, I. van der Berg, G. Böhmer, J. Wiemer, *Soc. Neurosci. Abstr.* **28**, 840.3 (2002).
21. S. L. Foote, J. H. Morrison, *Annu. Rev. Neurosci.* **10**, 67 (1987).
22. M. P. Kilgard, M. M. Merzenich, *Science* **279**, 1714 (1998).
23. P. R. Montague, P. Dayan, T. J. Sejnowski, *J. Neurosci.* **16**, 1936 (1996).
24. S. Bao, V. T. Chan, M. M. Merzenich, *Nature* **412**, 79 (2001).
25. C. M. Bütefisch *et al.*, *Ann. Neurol.* **51**, 59 (2002).
26. C. Baumgartner *et al.*, *Exp. Brain Res.* **87,** 641 (1991).
27. M. Scherg, H. Buchner, *Physiol. Meas.* **14** (Suppl. 4A), A35 (1993).
28. N. L. Golding, N. P. Staff, N. Spruston, *Nature* **418**, 326 (2002).

29. We thank G. Böhmer and D. Jancke for critical reading of earlier versions of the manuscript. Supported by the Deutsche Forschungsgemeinschaft (grant no. Di334/10-3 to H.D. and M.T. and grant no. Di334/15 to H.D.) and by a grant of the Heinrich-und-Alma-Vogelsang-Stiftung (P.S.). P.R. holds a scholarship from the International Graduate School for Neuroscience, Ruhr-University Bochum.

Supporting Online Material
www.sciencemag.org/cgi/content/full/301/5629/91/DC1
Materials and Methods
Fig. S1
References and Notes

8 April 2003; accepted 2 June 2003

Role of Raf in Vascular Protection from Distinct Apoptotic Stimuli

Alireza Alavi,* John D. Hood,* Ricardo Frausto, Dwayne G. Stupack, David A. Cheresh†

Raf kinases have been linked to endothelial cell survival. Here, we show that basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) differentially activate Raf, resulting in protection from distinct pathways of apoptosis in human endothelial cells and chick embryo vasculature. bFGF activated Raf-1 via p21-activated protein kinase–1 (PAK-1) phosphorylation of serines 338 and 339, resulting in Raf-1 mitochondrial translocation and endothelial cell protection from the intrinsic pathway of apoptosis, independent of the mitogen-activated protein kinase kinase–1 (MEK1). In contrast, VEGF activated Raf-1 via Src kinase, leading to phosphorylation of tyrosines 340 and 341 and MEK1-dependent protection from extrinsic-mediated apoptosis. These findings implicate Raf-1 as a pivotal regulator of endothelial cell survival during angiogenesis.

Vascular remodeling and neovascularization can be induced by a wide variety of cytokines and growth factors. In addition to promoting endothelial cell (EC) proliferation and invasion, angiogenic growth factors protect ECs from both intrinsic and extrinsic inducers of apoptosis. The intrinsic pathway is activated at the mitochondria in response to stress, such as nutrient deprivation or DNA damage, whereas the extrinsic pathway is induced by receptor binding to proapoptotic death ligands such as tumor necrosis factor–α (TNF-α) and Fas.

bFGF and VEGF are EC survival factors that activate two distinct signaling pathways leading to angiogenesis (*1–4*). Because Raf kinases have been shown to be essential to this process (*5–8*), we evaluated the mechanisms underlying the antiapoptotic functions of bFGF and VEGF and the role played by Raf kinases in this response. We exposed ECs to the individual growth factors and induced the cells to undergo apoptosis through either the intrinsic (stress) or extrinsic (receptor) pathway. Surprisingly, bFGF preferentially protected ECs from stress-mediated death, whereas VEGF was primarily effective against receptor-mediated apoptosis

Department of Immunology, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: cheresh@scripps.edu