early infection preserved CD4 cell counts and permitted the induction of persistent SHIV-specific cellular immune responses. Stronger responses against gag and nef proteins were observed in the early treatment cohort and were evident as early as day 25 after infection (gag, $P < 0.033$; nef, $P < 4 \times 10^{-5}$). These responses involved both $CD8^+$ and $CD4^+$ T cells (Table 1) and likely contributed to the robust antiviral responses observed during L-870812 treatment and to the control of virus replication after the discontinuation of therapy (Fig. 2). Neutralizing antibodies were minimal throughout the experimental period (*14*).

Therapy with L-870812 in the delayed treatment cohort did not either induce or increase cellular immune responses. However, the animals (01-R017 and 98-1053) that expressed the best outcomes both before and during therapy exhibited preexisting SHIV-specific cellular immune responses that correlated with their respective response to treatment (Fig. 3 and Table 1). Animal 98-0153 initiated therapy with the highest CD4 cell count (150 cells/μl) and SHIV-specific cellular immune response and was the only animal in the group to achieve and maintain vRNA below 10^2 copies/ml. Conversely, 99-0111, 99-0079, 99-0085, and 01-R023 did not develop virus-specific cellular immunity and had the least durable response to L-870812. The correlation of the overall apparent efficacy of L-870812 treatment between the two cohorts and among animals within the delayed treatment group with viral-specific cellular immune responses suggests that cellular immunity may facilitate the control of retroviral replication mediated by an effective chemotherapeutic agent such as L-870812.

Integrase inhibitors represent a new class of agents to treat HIV-1 infection in therapy-naïve patients and patients harboring viruses resistant to current antiretroviral agents. The antiviral activity of L-870812 in SHIV-89.6P–infected rhesus macaques provides evidence of in vivo efficacy for integrase inhibitors in retroviral infections, demonstrating that integrase inhibitors can be engineered with the appropriate properties required for an effective therapy to treat chronic HIV-1 infection. Oral administration of L-870812 was well tolerated, and the animals in this study exhibited no clinical signs of toxicity. In animals with detectable viremia, chronic treatment with L-870812 selected a viral variant with an integrase mutation (N155H) that exhibited lower replication capacity and reduced pathogenicity, supporting observations from in vitro studies with resistant isolates selected with related inhibitors that have suggested that integrase inhibitors may present a high genetic barrier to resistance development (*11*, *15*). Consistent with studies of human acute seroconverters and antiretroviral therapy in early HIV-1 disease (*16*, *17*), L-870812 therapy was most effective in animals that initiated treatment early in infection. Early treatment with L-870812 permitted the induction of substantial antiviral-specific cellular immunity. Immune status and viral load also affected the overall response to L-870812 in animals initiating therapy later in infection. These studies therefore suggest that cellular immunity may facilitate the durability of antiretroviral chemotherapy and provide evidence to support the use of vaccines to enhance and sustain the efficacy of antiretroviral therapy as well as the reevaluation of early intervention strategies for treating HIV-1 infection with improved antiviral regimens.

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20. We appreciate the technical support of P. Felock, K. Stillmock, A. Carella, G. Moyer, R. Newton, P. Sczerba, P. Reebeck, M. Washington, and K. Wright.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1098632/DC1
Materials and Methods
References

1 April 2004; accepted 23 June 2004
Published online 8 July 2004; 10.1126/science.1098632
Include this information when citing this paper.

Acquired Dendritic Channelopathy in Temporal Lobe Epilepsy

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Inherited channelopathies are at the origin of many neurological disorders. Here we report a form of channelopathy that is acquired in experimental temporal lobe epilepsy (TLE), the most common form of epilepsy in adults. The excitability of CA1 pyramidal neuron dendrites was increased in TLE because of decreased availability of A-type potassium ion channels due to transcriptional (loss of channels) and posttranslational (increased channel phosphorylation by extracellular signal-regulated kinase) mechanisms. Kinase inhibition partly reversed dendritic excitability to control levels. Such acquired channelopathy is likely to amplify neuronal activity and may contribute to the initiation and/or propagation of seizures in TLE.

Epileptic discharges correspond to highly synchronized and high-frequency activity of neurons. An augmented neuronal input-output relation is thought to be at the core of this increased excitability (*1*). Changes in the input-output relation of neurons may be caused by plastic changes on the synaptic level as reported in human and experimental epilepsy (*2*–*4*). Far less is known about changes of ion channels in the neuronal membrane, even though they control synaptic integration and intrinsic neuronal excitability. A number of rare genetic epilepsy syndromes are linked to mutations of ion channels (*5*), and acquired changes of somatic ion channels can occur after seizures (*6*, *7*). The fate of dendritic ion channels, however, has remained an enigma. As the main site for synaptic integration, neuronal dendrites could play a key role in seizure initiation and propagation (*8*–*10*). Dendrites contain a very high density of ion channels that can be targeted by antiepileptic drugs (*11*, *12*). Through their action on back-propagating action potentials (b-APs) (*13*, *14*), A-type K^+ channels are crucial modulators of information processing and synaptic plasticity in the dendrites. A reduction in A-type K^+ channel activity increases b-AP amplitude and promotes burst firing in a

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location-dependent manner (*13*, *15*). One useful measure of availability of A-type K^+ channels, as well as of dendritic excitability, is thus the amplitude of the b-AP (*16*, *17*).

With the use of whole-cell dendritic recordings in hippocampal CA1 pyramidal cells (*18*), we tested whether back-propagation was modified in pilocarpine-treated rats, an animal model for epilepsy that shares many pathophysiological similarities with TLE in humans (*19*). The amplitude of b-APs decreased with distance from the soma in sham (Fig. 1, A and B) ($n = 41$) as in control animals (*16*, *20*). In the proximal part of the dendritic tree (<200 μm), back-propagation is mostly active and b-APs have a large amplitude (*20*). In the distal part (>250 μm), back-propagation is mostly passive and b-APs have a small amplitude. In epileptic animals ($n = 62$), b-AP amplitudes were significantly larger than those in sham ($n = 50$) at distances greater than 250 μm from the soma (Fig. 1, A and B). Other features of back-propagation, including the AP initiation site and resting membrane potentials (RMPs), were not modified in epileptic animals (figs. S1 and S2).

What could be the mechanisms for the invasion of the dendritic tree by large-amplitude b-APs in TLE? Although back-propagation is decremental in control tissue, changing the availability of Na^+ and/or K^+ channels can switch back-propagation from passive to active (*21*, *22*). The switch occurs when the membrane potential crosses a threshold potential θ_x (Fig. 2C), which takes into account Na^+ and/or K^+ channel availability at any given distance x from the soma (*20*). To measure θ_x, current pulses were injected to the dendrites to achieve various membrane potentials (V_m). At hyperpolarized levels, b-AP amplitude was small (Fig. 1C). As the membrane was further depolarized, there was a sudden increase in amplitude until the peak of the b-AP reached a maximum value (Fig. 1C). The transition threshold θ_x is the membrane potential where the second derivative of the b-AP as a function of V_m is maximum (*20*). In the example shown in Fig. 1C (320 μm from the soma), b-AP had a small amplitude at RMP in the sham animal because $\theta_{320} >$ RMP (blue dots). In contrast, at the same distance in the epileptic animal, $\theta_{320} <$ RMP and b-AP had a large amplitude at RMP. The distribution of θ_x along the dendritic tree in sham animals (Fig. 1D) ($n = 29$) was similar to that found in controls (*20*), i.e., $\theta_x >$ RMP for $x > 260$ μm and $\theta_x <$ RMP for $x < 260$ μm. However, the distribution of θ_x was shifted toward more negative values in epileptic animals (Fig. 1D) ($n = 28$), enabling active b-APs in TLE in the dendritic region that normally experienced passive b-APs in sham animals.

What could be the mechanisms responsible for the modification of θ? Because b-AP amplitude and θ are very sensitive to A-type K^+ channel availability (*17*), a decrease in A-type

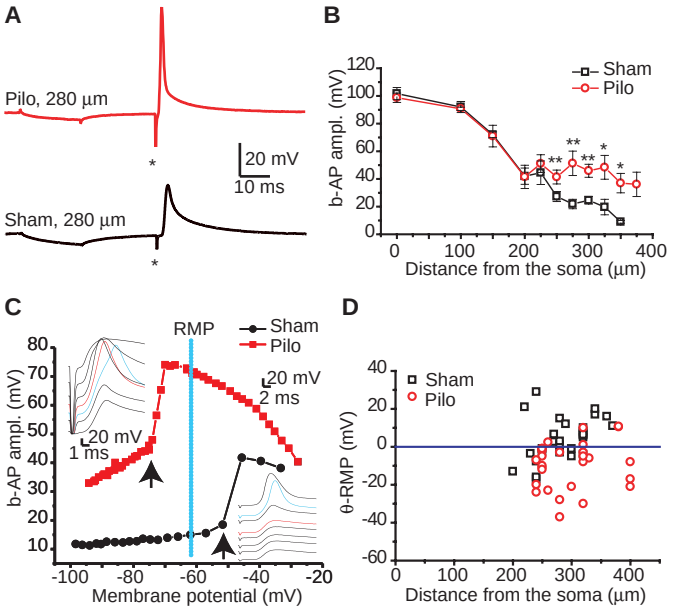

Fig. 1. Large-amplitude b-APs invade the distal part of the dendritic tree in TLE because of a modification of the threshold that determines the transition from passive to active back-propagation. (**A**) b-AP amplitude was increased in the epileptic animal. Asterisks indicate a stimulus artifact. (**B**) Distribution of b-AP amplitudes measured at RMP as a function of distance from the soma (25-μm bins) in sham ($n = 50$) and epileptic ($n = 62$) animals. Whereas b-AP amplitude decreased progressively in sham animals, b-AP amplitudes remained large and constant at distances > 200 μm in epileptic animals (45 ± 2 mV, $n = 53$, and no statistical difference between any bins). The difference with sham animals became significant >250 μm (double asterisks, $P < 0.01$; single asterisk, $P < 0.05$). (**C**) Variation of b-AP amplitude as a function of membrane potential [black dots, sham; red squares, pilo (epileptic); blue dotted trace, RMP of –59 mV for sham and –64 mV for pilo]. Arrows indicate θ, the threshold for the transition between passive and active back-propagation. Because $\theta_{sham} > RMP_{sham}$ whereas $\theta_{epileptic} < RMP_{pilo}$, back-propagation at RMP was passive in the sham animal and active in the pilo animal. Insets display b-APs measured at various membrane potentials from hyperpolarized (–90 mV, bottom traces) to depolarized (–30 mV, top traces) potentials. As the membrane was depolarized, there was a sudden increase in amplitude (blue trace). Red trace, b-AP at RMP. (**D**) Difference between θ and RMP at each recording site as a function of the distance from the soma (black squares, sham, $n = 29$; red dots, pilo, $n = 28$). Above the blue line, where $\theta_x = RMP_x$ at any distance x from the soma, b-APs have a small amplitude. Below the blue line, $\theta_x < RMP_x$ and b-APs have a large amplitude. In the distal part of the dendritic tree (>250 μm), most values are above the blue line in the sham animals. By contrast, they are below the blue line in the epileptic animals.

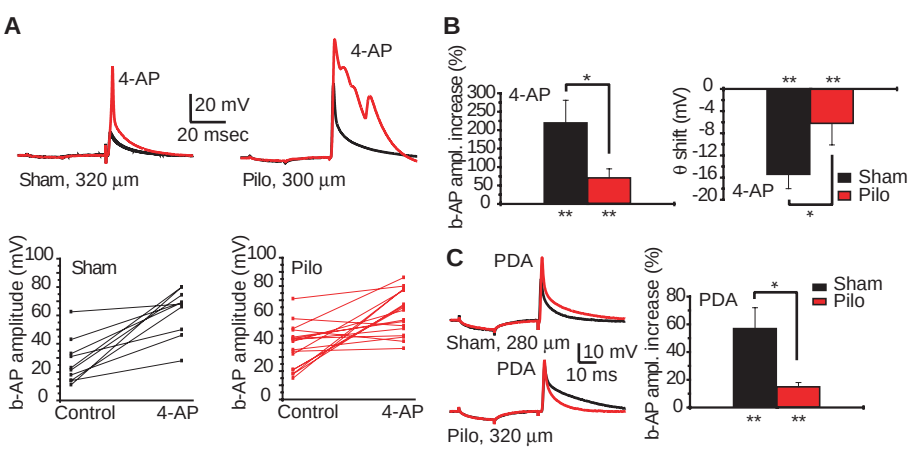

Fig. 2. The control of back-propagation by A-type K^+ channels was decreased in TLE. (**A**) Application of the K^+ channel blocker 4-AP (5 mM) increased b-AP amplitude in sham and epileptic animals. Typical examples are displayed at the top. The amplitude at the peak of the b-AP was similar in sham (63 ± 6 mV, $n = 10$, black squares) and epileptic (61 ± 4 mV, $n = 17$, red dots) animals ($P = 0.83$ between sham and epileptic) in the presence of 4-AP (bottom). (**B**) The 4-AP–induced increase in amplitude was statistically greater in sham than in epileptic animals (double asterisks, $P < 0.0003$; single asterisk, $P < 0.01$; left). The presence of 4-AP shifted θ toward more negative membrane potentials, but the shift was statistically greater in sham than in epileptic (double asterisks, $P < 0.0007$; single asterisk, $P < 0.01$; right). These results indicate that fewer A-type K^+ channels are available in epileptic animals in the absence of 4-AP. (**C**) Application of the PKC activator PDA (10 μM) increased b-AP amplitude by decreasing the availability of A-type K^+ channels. The increase in amplitude was statistically smaller in epileptic ($n = 5$) as compared to sham ($n = 5$) animals (double asterisks, $P < 0.01$; single asterisk, $P < 0.05$; right), suggesting an increased basal level of phosphorylation of A-type K^+ channels in epileptic animals.

K^+ function could result in increased back-propagation and a shift of θ (*20*). Although b-AP amplitude is smaller in sham than in epileptic animals at similar dendritic locations, blocking A-type K^+ channels with 4-AP (5 mM) had a larger effect in sham (Fig. 2, A and B) (220 ± 61% of control for $n = 10$) than in epileptic (71 ± 24% for $n = 17$) animals. Blocking A-type K^+ channels induced a shift of θ toward more negative values, which was significantly larger in epileptic (15.5 ± 2.5 mV for $n = 5$) than in sham (6.2 ± 3.8 mV for $n = 5$) animals (Fig. 2B). These results suggest that the control of b-AP amplitude by K^+ channel is decreased in TLE. Interestingly, the relation between b-AP max *dV/dt* and b-AP amplitude in the presence of 4-AP was similar in sham (slope of 1.0, $r = 0.8$, and $n = 10$) and epileptic (slope of 0.9, $r = 0.9$, and $n = 17$) animals, suggesting that ion channels that control the rising phase of b-APs (e.g., Na^+ channels) are not modified in TLE. Collectively, these results indicate that a decreased availability of 4-AP–sensitive K^+ channels most likely accounts for the increased dendritic excitability found in TLE. The reduced availability of these channels may be caused by reduction in A-type K^+ channel density, altered modulation of the channel protein by phosphorylation, or both.

A-type K^+ channels in CA1 pyramidal cell dendrites are modulated by protein kinase C (PKC). This kinase causes phosphorylation of Kv4.2 channels (*17*) and shifts the activation curve of A-type K^+ channels toward more positive values, thereby increasing b-AP amplitude (*17*, *23*). If phosphorylation of A-type K^+ channels is increased in TLE, PKC activation should have smaller effects on back-propagation in TLE as compared to sham. Indeed, application of 10 μM phorbol diacetate (PDA), a PKC activator, had a smaller effect in epileptic (15 ± 3% for $n = 5$) compared to sham (57 ± 15% for $n = 5$) animals (Fig. 2C). We next inhibited phosphorylation by application of the broad spectrum protein kinase inhibitor H7. In sham animals, bath application of 300 μM H7 decreased b-AP amplitude by 12 ± 4% (Fig. 3, A and C) ($n = 5$) and caused a shift of θ toward more positive values (Fig. 3, A and C) (7.4 ± 0.4 mV for $n = 5$), consistent with the basal level of phosphorylation of K^+ channels reported previously (*17*). In epileptic animals, H7 decreased the amplitude of b-APs by 24 ± 7% (Fig. 3, B and C) ($n = 8$). The θ was shifted toward more positive values than in sham animals (Fig. 3, B and C) (14.5 ± 3.6 mV for $n = 5$). In epileptic animals, the effect of H7 on b-AP amplitude depended upon the initial value of θ before H7 application. If the difference between θ and RMP before H7 was greater than 14.5 mV (on average), back-propagation remained active (left of θ) (Fig. 3B, right). If the difference was smaller than 14.5 mV, back-propagation became weak (Fig. 3B, left). This suggests that additional modifications may be at play, such as a reduction in A-type K^+ channel density.

Kv4.2 channels are one of the final effectors of extracellular signal-regulated kinase (ERK), which is phosphorylated and activated downstream of PKC (*17*). We applied U0126, an inhibitor of mitogen-activated protein kinase (MEK), to block ERK activation and prevent the phosphorylation of Kv4.2 channels (*17*). Bath application of 10 μM U0126 for 30 min decreased b-AP amplitudes by 21.8 ± 5.9% ($n = 7$) and by 41.2 ± 10.2% ($n = 11$) in sham and epileptic animals, respectively (Fig. 4). The θ was shifted toward more positive values by 5.1 ± 2.0 mV ($n = 7$) and 19.1 ± 5.4 mV ($n = 11$) in sham and epileptic animals, respectively (Fig. 4). Targeting kinase inhibition more selectively in the ERK pathway thus resulted in a reduction of dendritic excitability comparable to that obtained with the kinase inhibitor H7 (fig. S3).

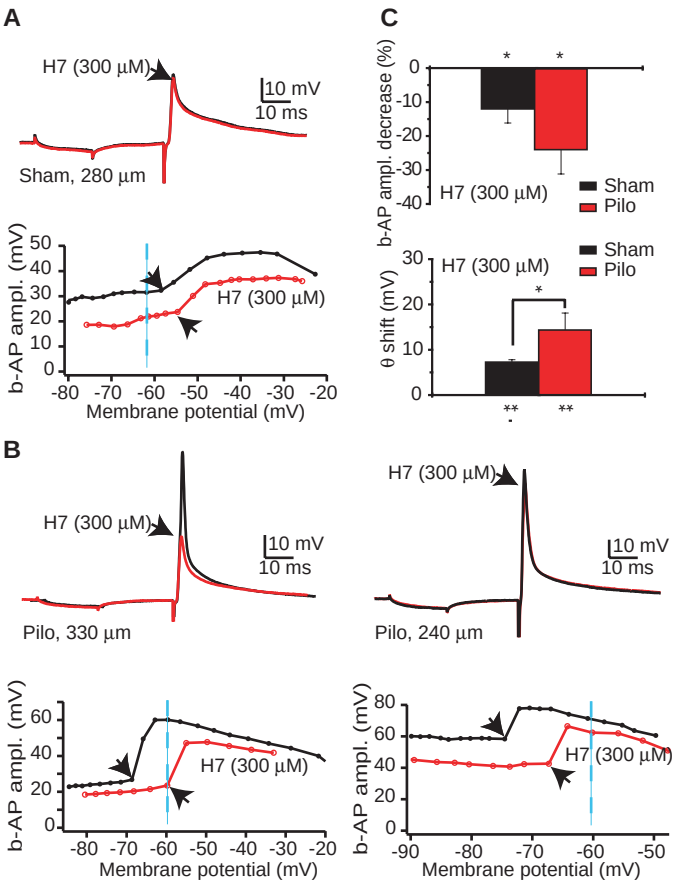

Fig. 3. Preventing A-type K^+ channel phosphorylation partly restores back-propagation in TLE. (**A**) Application of H7 (300 μM) slightly decreased b-AP amplitude and shifted the transition threshold (arrows) toward more positive values in sham animals, consistent with a small basal level of phosphorylation of A-type K^+ channels. (**B**) H7 had either a large or a small effect on b-AP amplitude, according to the initial value of θ (before H7 application). If H7 shifted θ at a potential greater than RMP, b-AP was considerably reduced (left) when back-propagation was switched back from active to passive. If the H7-induced shift left θ more negative than RMP, b-AP was slightly reduced (right), as in sham animals, and back-propagation remained active. (**C**) H7 significantly decreased b-AP amplitude in sham ($n = 5$) and epileptic ($n = 8$) animals (single asterisk, $P < 0.05$; top), but the difference in the decrease between sham and epileptic animals was not statistically significant ($P = 0.07$). The H7-induced shift of θ toward more positive values was significantly greater in epileptic than in sham animals (double asterisks, $P < 0.002$; single asterisk, $P < 0.05$; bottom), consistent with an increased level of phosphorylation of A-type K^+ channels in TLE.

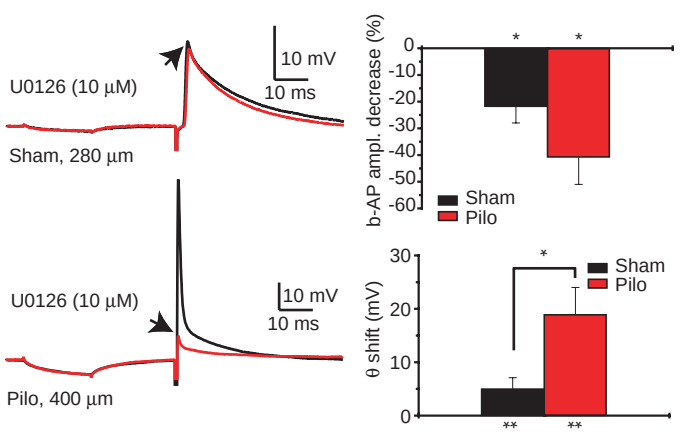

Fig. 4. ERK inhibition partly restores back-propagation in TLE. Application of the MEK inhibitor U0126 (10 μM) shifted the transition threshold to more positive values and decreased b-AP amplitudes. The shift of θ was greater in epileptic ($n = 11$) than in sham ($n = 7$) animals (double asterisks, $P < 0.0001$; single asterisk, $P < 0.05$).

We next directly examined the expression of Kv4.2 channels as well as their phosphorylation status. With the use of an antibody that selectively recognizes Kv4.2 when phosphorylated at the ERK sites (*24*), we found a significant increase in the percent of ERK phosphorylated-Kv4.2 (fig. S4A) and a significant decrease in total Kv4.2 channel protein (fig. S4B) in epileptic as compared to sham animals. With the use of quantitative real-time reverse transcription polymerase chain reaction, we found a significant reduction in Kv4.2 mRNA amounts in epileptic compared to control animals (fig. S4, C and D).

Our results provide evidence that dendritic excitability in CA1 pyramidal neurons is increased in an animal model of TLE during the chronic phase of epilepsy. A decrease in availability of A-type K^+ channels largely contributes to this phenomenon because of a partial loss of Kv4.2 K^+ channels and increased ERK phosphorylation of the remaining ones, consistent with the increased amounts of PKC and ERK activation after seizures (*25*, *26*). Other channel types may also contribute to enhanced dendritic excitability. A transcriptional up-regulation of T-type Ca^{2+} channels has been reported in the soma of CA1 pyramidal cells in epileptic animals (*7*). Although our results suggest that the reported increased dendritic excitability is not due to a change of availability of transient Na^+ channels, a contribution of persistent Na^+ channels cannot be ruled out (*27*). Modifications of other somatic channels such as I_h have been reported in seizure models (*6*), but these channels do not appear to contribute much to b-AP amplitude (*28*).

Down-regulation of A-type K^+ channel function in pyramidal neuron dendrites will increase neuronal excitability in multiple ways. The increased amplitude of back-propagating APs will lead to greater calcium influx through voltage-gated channels, boosting dendritic excitatory postsynaptic potentials (EPSPs), particularly those arriving distally from the entorhinal cortex (*3*), and increasing EPSP-spike coupling (*29*) through inactivation of dendritic K^+ channels. This increase in excitability is likely to lower the threshold for seizure initiation in the hippocampus, a frequent site of focal seizure onset in human temporal lobe epilepsy.

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30. This work was supported by INSERM; NIH grants NS39943, NS37444, MH44754, and MH48432; Ligue Française Contre l'Epilepsie; Sanofi Synthélabo; and Fondation Simone et Cino Del Duca. H.B. was supported by SFB-TR3 subproject C2 and Heisenberg stipend of the Deutsche Forschungs gemeinschaft. We thank M. Esclapez for helping to set up morphology, R. Gray for computer help, and P. Bregestovski for helpful comments on the manuscript. C.B. also thanks Y. Liang and other members of the lab for help with animal dissections.

Supporting Online Material

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Materials and Methods

23 February 2004; accepted 24 May 2004