$GABA_B$ autoreceptors regulate the induction of LTP

Ceri H. Davies*†, Sarah J. Starkey*‡, Mario F. Pozza*‡ & Graham L. Collingridge*†§

* Department of Pharmacology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK
and † Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
‡ Present addresses: Department of Neuropharmacology, Glaxo Research Ltd, Ware, Herts SG12 ODJ, UK (S.J.S.); Ciba-Geigy Ltd, Research Department, Pharmaceuticals Division, K-125.7.09+7.13, CH-4002 Basle, Switzerland (M.F.P.)
§ To whom correspondence should be addressed at the University of Birmingham

UNDERSTANDING the mechanisms involved in long-term potentiation (LTP) should provide insights into the cellular and molecular basis of learning and memory in vertebrates[1]. It has been established that in the CA1 region of the hippocampus the induction of LTP requires the transient activation of the *N*-methyl-D-aspartate (NMDA) receptor system[2]. During low-frequency transmission, significant activation of this system is prevented by γ-aminobutyric acid (GABA) mediated synaptic inhibition[3,4] which hyperpolarizes neurons into a region where NMDA receptor-operated channels are substantially blocked by Mg^{2+} (refs. 5, 6). But during high-frequency transmission, mechanisms are evoked that provide sufficient depolarization of the postsynaptic membrane to reduce this block[7] and thereby permit the induction of LTP. We now report that this critical depolarization is enabled because during high-frequency transmission GABA depresses its own release by an action on $GABA_B$ autoreceptors, which permits sufficient NMDA receptor activation for the induction of LTP. These findings demonstrate a role for $GABA_B$ receptors in synaptic plasticity.

Fatigue of synaptic inhibition during tetanic stimulation is a well established phenomenon[8,9] but the mechanism underlying this process has only become clearer with the development of $GABA_B$ antagonists. As shown in Fig. 1*a*, if two shocks are delivered 200 ms apart to a monosynaptic inhibitory pathway, the second synaptic response is greatly depressed. This effect is probably due to a presynaptic action of GABA on inhibitory terminals[10,11]. A new $GABA_B$ receptor antagonist, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP 35348; ref. 12), can completely block this fatigue, but high doses (1 mM) are required—about 10 times more than is needed to abolish the postsynaptic $GABA_B$ receptor-mediated synaptic response (Fig. 1*a*). Fatigue of synaptic inhibition may be an explanation for why LTP can be induced by brief trains of stimuli delivered at frequencies similar to the theta rhythm[13,14]. We therefore investigated the effects of CGP 35348 on synaptic inhibition using one such 'primed-burst' paradigm; four shocks delivered at 100 Hz, preceded by 200 ms by a single shock (Fig. 1*b*). The $GABA_B$ antagonist, by reducing the fatigue initiated by the priming pulse, allowed more efficient summation of the $GABA_A$ receptor-mediated inhibitory postsynaptic currents (i.p.s.cs) during the subsequent high-frequency burst.

Although 1 mM CGP 35348 was necessary to block completely paired-pulse depression, this effect was selective in that $GABA_A$ receptor-mediated i.p.s.cs were not directly affected to any significant extent (Fig. 1). But as it is not known how selective this $GABA_B$ antagonist is (at 1 mM) towards other presynaptic receptors, we compared the effect of CGP 35348 on depressions of field excitatory postsynaptic potentials (e.p.s.ps) induced by baclofen, 2-chloroadenosine, and carbachol. CGP 35348 rapidly abolished the depression evoked by the $GABA_B$ receptor agonist baclofen but had no effect on depressions evoked by the other agonists (Fig. 2*a*); it did not affect the amplitude of the field e.p.s.ps *per se* (the peak amplitudes before and during the application of CGP 35348 were 1.3 ± 0.2 and 1.4 ± 0.4 mV ($n = 9$), respectively). CGP 35348 did, however, reverse the depression

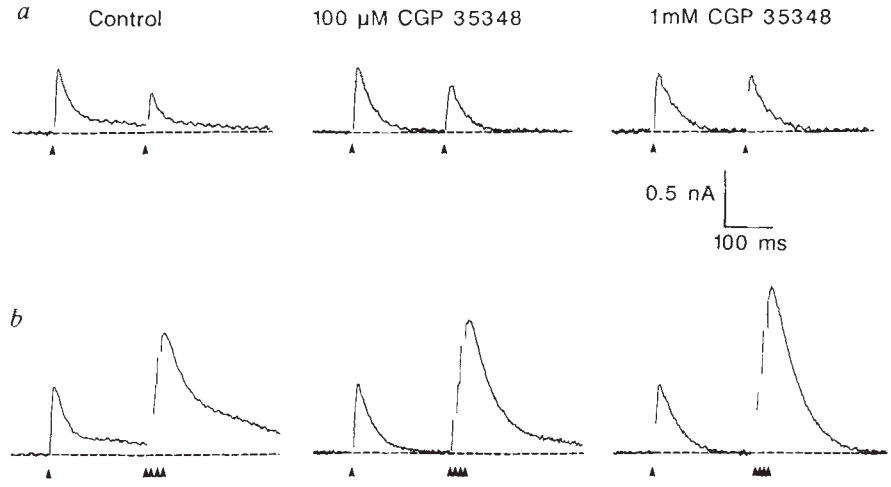

FIG. 1 CGP 35348 blocks the $GABA_B$ receptor-mediated i.p.s.c. and reverses paired-pulse (*a*) and primed-burst (*b*) induced depressions of monosynaptically-activated $GABA_A$ receptor-mediated i.p.s.cs. *a*, Examples of i.p.s.cs evoked by two identical shocks 200 ms apart in a voltage-clamped pyramidal neuron, held at −55 mV. Traces show (from left to right) paired-pulse depression under control conditions, blockade of the $GABA_B$ receptor-mediated synaptic component with partial reversal of paired-pulse depression (at 100 μM), and almost complete reversal of paired-pulse depression (at 1 mM) of $GABA_A$ receptor-mediated i.p.s.cs. In 6 similar experiments, CGP 35348 depressed the $GABA_B$ receptor-mediated i.p.s.c. by 93 ± 1 and by 94 ± 6%, at 100 μM and 1 mM, respectively ($P > 0.05$). In the same experiments, paired-pulse depression of the $GABA_A$ receptor-mediated i.p.s.c. was reversed by 36 ± 4 and by 92 ± 2%, respectively ($P < 0.0005$). (See ref. 11 for characterization of monosynaptic i.p.s.cs). In *b*, i.p.s.cs evoked in the same pyramidal neuron as in *a* by a primed-burst tetanus. In five similar experiments the amplitude of the peak amplitude of the i.p.s.c., evoked by the high-frequency burst, in the presence of 100 μM and 1 mM CGP 35348 were 121 ± 7 and 164 ± 10% of control, respectively. In this and subsequent figures synaptic records are averages of 3–5 individual responses and the point of each stimulus is marked by a small arrowhead. Stimulation artefacts are blanked for clarity.

METHODS. Rat hippocampal slices (400 μm thick) were prepared using standard techniques and maintained in an interface chamber at 30–32°C perfused with a medium containing (mM): NaCl, 124; KCl, 3; $NaHCO_3$, 26; $CaCl_2$, 2; $MgSO_4$, 1; D-glucose, 10; NaH_2PO_4, 1.25, bubbled with a 95% O_2/5% CO_2 mixture. Intracellular and extracellular microelectrodes were filled with CH_3COOK or potassium methyl sulphate (2–3 M, 20–60 MΩ), and NaCl (4 M, 2–6 MΩ) respectively. Intracellular single-electrode, voltage-clamped recordings (switching frequency 6–12 kHz) were made from CA1 pyramidal neurons using an Axoclamp-2 amplifier. Monosynaptic i.p.s.cs were evoked in the presence of 20 μM CNQX and 40 μM D-AP5 to block all excitatory synaptic responses, as described previously[11]. Field e.p.s.ps were recorded extracellularly from stratum radiatum in the CA1 region. All experiments involved stimulation (width 20 μs, 5–20 V) of the Schaffer collateral-commissural afferents at 0.033 Hz. In the LTP experiments the test e.p.s.p. size was adjusted to ~50% of maximum. LTP was induced using a single 'primed-burst' protocol which comprised four pulses delivered at 100 Hz preceded by 200 ms by a single pulse. (All five shocks were at the test intensity but their duration was increased to 200 μs). Slope measurements were made between 20% and 80% of the peak e.p.s.p. amplitude. Drugs were applied by bath perfusion. Data are presented as means ±s.e.m. and statistical significance was assessed using Student's *t*-tests.

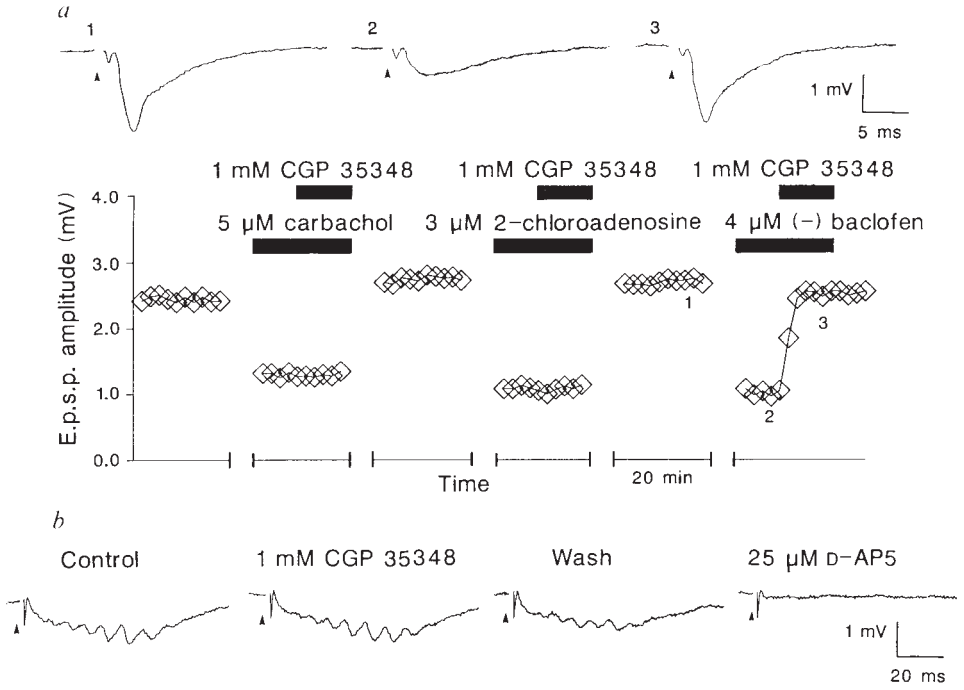

FIG. 2 Selectivity of 1 mM CGP 35348. *a*, The graph illustrates extracts from a single experiment showing the effects of carbachol, 2-chloroadenosine and (−)-baclofen (duration of applications denoted by a solid bar) on the amplitude of the field e.p.s.p. CGP 35348 (co-applied with the agonist) rapidly reversed the (−)-baclofen-induced depression without any effect on the depressions induced by the other agonists. Identical results were obtained in a total of six slices using carbachol (4–5 μM; $n=3$), 2-chloroadenosine (1–3 μM; $n=3$) and (−)-baclofen (3–4 μM; $n=3$). Each point is the average of four consecutive measurements (wash-in and wash-out of drugs not shown). Traces of reversal of the baclofen-induced depression are shown for the points indicated by 1–3. *b*, Field e.p.s.ps, with superimposed multiple population spikes, were evoked in medium containing picrotoxin (100 μM) and CNQX (10 μM). Traces illustrate a typical example of records obtained (from left to right) in control, in the presence of CGP 35348, 1 h after washout of CGP 35348 and in the presence of D-AP5. Note that CGP 35348 did not reduce the D-AP5-sensitive component of the response. (CGP 35348 caused a slight increase in the latter part of the response, presumably as a result of blockade of the $GABA_B$ receptor-mediated i.p.s.p.). Identical results were obtained in three separate experiments.

of field e.p.s.ps induced by 5–10 mM GABA in the presence of 100 μM picrotoxin ($n=3$). This shows that CGP 35348 is effective against the likely neurotransmitter that acts at the $GABA_B$ receptors responsible for fatigue of synaptic inhibition. We next established whether CGP 35348 directly affected the NMDA receptor system by determining its effect on field e.p.s.ps recorded either in medium containing low magnesium[15] ($n=3$), or in medium containing picrotoxin and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) ($n=3$). CGP 35348 (1 mM) had no effect on the NMDA receptor-mediated component of these responses, as defined using the selective NMDA antagonist D-2-amino-5-phosphonopentanoate (D-AP5) (Fig. 2*b*).

The priming pulse, by reducing synaptic inhibition, ought to facilitate the summation of NMDA receptor-mediated components during the high-frequency burst. Presumably therefore, CGP 35348 should, by facilitating the $GABA_A$ receptor-mediated synaptic inhibition, reduce the synaptic activation of NMDA receptor-mediated e.p.s.ps during the burst. Figure 3 compares the effects of CGP 35348 and D-AP5 applied singularly or together on field e.p.s.ps evoked by the primed-burst protocol. Under control conditions, during and following the latter part of the burst, there is a slow component which is not seen in the presence of D-AP5. This NMDA receptor-mediated component is reduced by CGP 35348.

Finally we determined the extent to which this effect on the NMDA receptor system could influence the induction of LTP. In addition to testing the effects of CGP 35348 at 1 mM, we used a tenfold lower concentration; this mainly blocks the postsynaptic activation of $GABA_B$ receptors (see Fig. 1*a*). Hence we could estimate the involvement of presynaptic $GABA_B$ recep-

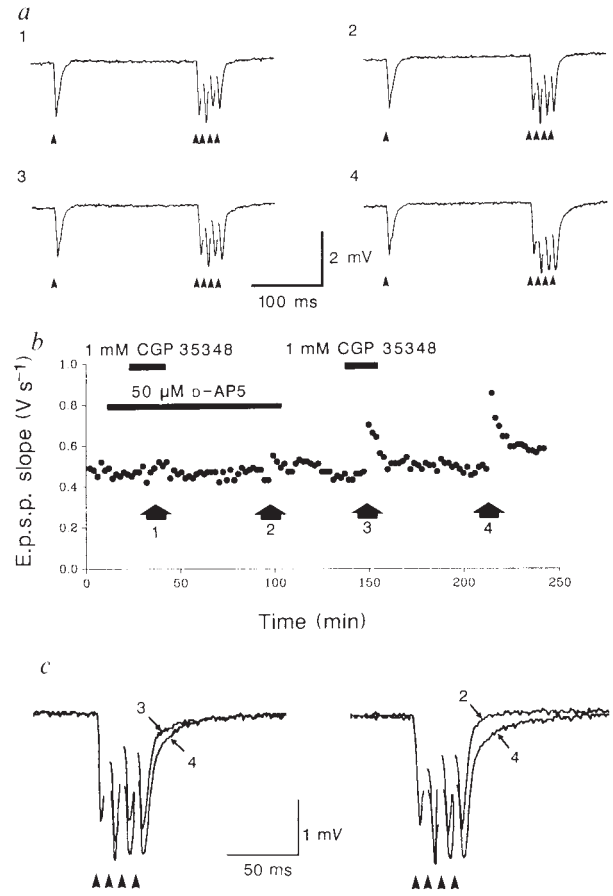

FIG. 3 CGP 35348 reduces the size of an NMDA receptor-mediated e.p.s.p. during a primed-burst tetanus. *a*, Traces 1–4 are the field e.p.s.ps evoked in a single experiment by primed-burst tetani delivered under the conditions shown in *b*. *b*, The slope of the field e.p.s.p. (average of four successive responses) is plotted for the duration of the experiment where primed-burst tetani were delivered sequentially in the presence of (1) a combination of CGP 35348 and D-AP5, (2) D-AP5 alone, (3) CGP 35348 alone and (4) in the absence of any antagonists. *c*, Superimposition of field e.p.s.ps evoked by the last four stimuli of the primed-burst tetani to illustrate the effects of CGP 35348 (left-hand trace) and D-AP5. Similar results were obtained in three separate experiments.

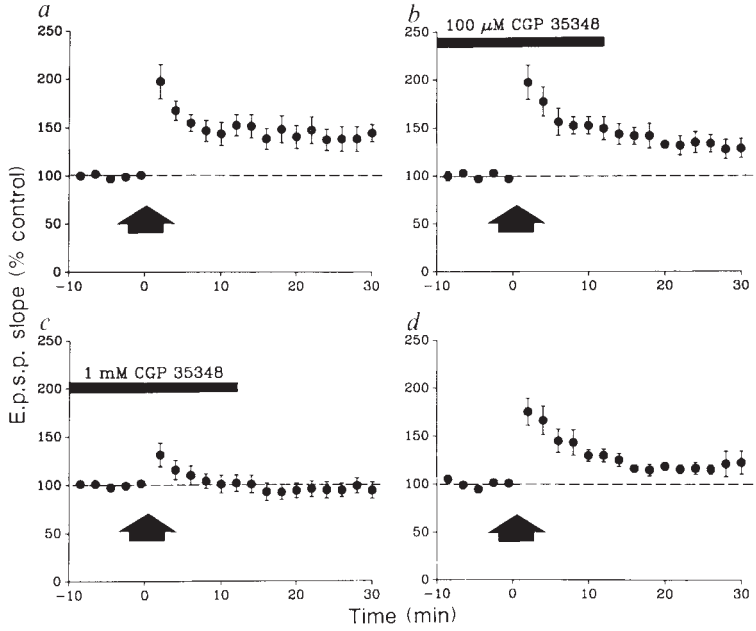

FIG. 4 CGP 35348 blocks the induction of LTP. *a*, Normalized pooled data for the change of field e.p.s.p. slope plotted as a function of time for control slices ($n=16$) induced by a primed-burst tetanus. *b*, Effects of 100 μM CGP 35348 on primed-burst induced LTP in a second population of slices ($n=8$). *c*, Effects of 1 mM CGP 35348 on primed-burst induced LTP in a third population of slices ($n=8$). *d*, LTP induced in the same slices following wash-out of 1 mM CGP 35348 ($n=8$).

tors in LTP. As shown in Fig. 4, the induction of primed-burst LTP is blocked by CGP 35348 at 1 mM but it is not significantly affected at 100 μM. To ensure that the blockade of LTP was due to prevention of fatigue of $GABA_A$ receptor-mediated inhibition rather than to some unrelated effect of CGP 35348, we tested the effects of the $GABA_B$ antagonist on the induction of LTP in the presence of picrotoxin. As expected for a block that was due to indirect potentiation of $GABA_A$ receptor-mediated inhibition, CGP 35348 did not affect LTP induced under these conditions (using a single train of four shocks, 100 Hz, test intensity, six controls versus six CGP 35348-treated slices).

Our data demonstrate that activation of $GABA_B$ receptors is necessary for the induction of LTP under standard experimental conditions. There are several sites in the CA1 region of the hippocampus where these receptors could be located. The $GABA_B$ receptors on the terminals of the excitatory fibres[16] are, as we show here, sensitive to CGP 35348. But these are unlikely to be the critical receptors as CGP 35348 had little or no direct effect on e.p.s.ps. Furthermore, if these receptors were to be activated by the primed-burst protocol, then the induction of LTP should be depressed, because baclofen inhibits the NMDA receptor-mediated component of synaptic transmission[17]; thus CGP 35348 would enhance rather than block this process. The importance of the $GABA_B$ receptors on inhibitory terminals[11] relative to those located postsynaptically on CA1 neurons[18] could be distinguished because CGP 35348, like other $GABA_B$ antagonists[11,19], preferentially inhibits the effects of postsynaptic $GABA_B$ receptor activation. As 100 μM CGP 35348 almost abolished the $GABA_B$ receptor-mediated i.p.s.c. without affecting the induction of LTP, the activation of postsynaptic $GABA_B$ receptors is unlikely to be necessary for the initiation of this process. Indeed, blockade of $GABA_B$-, like $GABA_A$-, receptor-mediated i.p.s.ps[20–22] can facilitate the induction of LTP, because elimination of their hyperpolarizing influence enhances the expression of the NMDA receptor-mediated conductance[3,4]. The additional effect of the higher dose of CGP 35348, which was required to block the induction of LTP, was a much greater reversal of inhibitory synaptic fatigue. This fatigue is believed to be due to GABA feeding back and inhibiting its own release through an action on $GABA_B$ receptors[10,11,23]; therefore a $GABA_B$ 'autoreceptor' is probably the site at which CGP 35348 acts to block the induction of LTP.

On the basis of these data, we propose that during high-frequency stimulation a critical factor for the induction of LTP is fatigue of synaptic inhibition, brought about by the activation of $GABA_B$ autoreceptors. The decreased level of hyperpolarization during high-frequency transmission reduces the extent to which NMDA receptor-operated channels are blocked by Mg^{2+}; this then allows NMDA receptor-mediated synaptic components to summate sufficiently to induce LTP. A factor that may facilitate this process is summation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor-mediated synaptic components; however, this is not an absolute requirement as LTP can be induced at a time when AMPA receptors are blocked pharmacologically[24,25]. Alterations in ionic activities, for example an increase in extracellular K^+, may also facilitate the induction of LTP; this contribution would be expected to increase with the number of shocks in the burst. The primed-burst protocol we have used mimicks physiological patterns of activation. As the LTP that it induces can be blocked completely by CGP 35348, $GABA_B$ autoreceptors may have an essential permissive role in certain forms of synaptic plasticity in the vertebrate central nervous system. □

Received 19 September; accepted 12 December 1990.

1. Bliss, T. V. P. & Lynch, M. A. *Neurol Neurobiol.* **35,** 3–72 (1988).
2. Collingridge, G. L., Kehl, S. J. & McLennan, H. *J. Physiol., Lond.* **334,** 33–46 (1983).
3. Dingledine, R., Hynes, M. A. & King, G. L. *J. Physiol., Lond.* **380,** 175–189 (1986).
4. Collingridge, G. L., Herron, C. E. & Lester, R. A. J. *J. Physiol., Lond.* **399,** 283–300 (1988).
5. Ascher, P. & Nowak, L. *J. Physiol., Lond.* **399,** 247–266 (1988).
6. Mayer, M. L. & Westbrook, G. L. *J. Physiol., Lond.* **361,** 65–90 (1985).
7. Collingridge, G. L., Herron, C. E. & Lester, R. A. J. *J. Physiol., Lond.* **399,** 301–312 (1988).
8. Ben-Ari, Y., Krnjevic, K. & Reinhardt, W. *Can. J. Physiol. Pharmacol.* **57,** 1462–1466 (1979).
9. McCarren, M. & Alger, B. E. *J. Neurophysiol.* **53,** 557–571 (1985).
10. Harrison, N. L. *J. Physiol., Lond.* **422,** 433–446 (1990).
11. Davies, C. H., Davies, S. N. & Collingridge, G. L. *J. Physiol., Lond.* **424,** 513–531 (1990).
12. Olpe, H.-R. *et al. Eur. J. Pharmac.* **187,** 27–38 (1990).
13. Larson, J. & Lynch, G. *Brain Res.* **441,** 111–118 (1988).
14. Diamond, D. M., Dunwiddie, T. V. & Rose, G. M. *J. Neurosci.* **8,** 4079–4088 (1988).
15. Coan, E. J. & Collingridge, G. L. *Neurosci. Lett.* **53,** 21–26 (1985).
16. Lanthorn, T. H. & Cotman, C. W. *Brain Res.* **225,** 171–178 (1981).
17. Randall, A. D., Schofield, J. G., Davies, C. H. & Collingridge, G. L. *J. Physiol., Lond.* **426,** 51P (1990).
18. Dutar, P. & Nicoll, R. A. *Nature* **332,** 156–158 (1988).
19. Dutar, P. & Nicoll, R. A. *Neuron* **1,** 585–591 (1988).
20. Wigström, H. & Gustafsson, B. *Nature* **301,** 603–604 (1983).
21. Mott, D. D., Lewis, D. V., Ferrari, C. M., Wilson, W. A. & Swartzwelder, H. S. *Neurosci. Lett.* **113,** 222–226 (1990).
22. Olpe, H.-R. & Karlsson, G. *Naunyn-Schmiedebergs Archs Pharmak.* **342,** 194–197 (1990).
23. Deisz, R. A. & Prince, D. A. *J. Physiol., Lond.* **412,** 513–542 (1989).
24. Kauer, J. A., Malenka, R. C. & Nicoll, R. A. *Neuron* **1,** 911–917 (1988).
25. Muller, D., Joly, M. & Lynch, G. *Science* **242,** 1694–1697 (1988).

ACKNOWLEDGEMENTS. We thank J. C. Watkins for gifts of CNQX and D-AP5, and the MRC for supporting this work. C. H. Davies is an A. J. Clark scholar.