10. Kellman, P. J. & Shipley, T. F. *Cognitive Psychology* **23,** 141–221 (1991).
11. Treisman, A. *Q. J. exp. Psychol.* **14,** 23–37 (1962).
12. Barlow, H. B., Blakemore, C. & Pettigrew, J. D. *J. Physiol., Lond.* **193,** 327–342 (1967).
13. Poggio, G. F. & Fischer, B. *J. Neurophysiol.* **40,** 1392–1407 (1977).
14. Takeichi, H., Watanabe, T. & Shimojo, S. *Perception* **21,** 177–184 (1992).

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Active propagation of somatic action potentials into neocortical pyramidal cell dendrites

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THE dendrites of neurons in the mammalian central nervous system have been considered as electrically passive structures which funnel synaptic potentials to the soma and axon initial segment, the site of action potential initiation[1,2]. More recent studies, however, have shown that the dendrites of many neurons are not passive, but contain active conductances[3,4]. The role of these dendritic voltage-activated channels in the initiation of action potentials in neurons is largely unknown. To assess this directly, patch-clamp recordings were made from the dendrites of neocortical pyramidal cells in brain slices. Voltage-activated sodium currents were observed in dendritic outside-out patches, while action potentials could be evoked by depolarizing current pulses or by synaptic stimulation during dendritic whole-cell recordings. To determine the site of initiation of these action potentials, simultaneous whole-cell recordings were made from the soma and the apical dendrite or axon of the same cell. These experiments showed that action potentials are initiated first in the axon and then actively propagate back into the dendritic tree.

Patch-clamp recordings from the apical dendrite of layer V pyramidal neurons revealed that the dendritic membrane of these cells contains voltage-activated channels. In response to step depolarizations from a negative holding potential, rapidly inactivating inward currents were observed in outside-out patches excised up to 530 μm from the soma. As these inward currents were blocked by tetrodotoxin (TTX), they presumably represent the activation of dendritic voltage-activated Na^+ channels (Fig. 1*a*). Despite considerable variability, the peak Na^+ current in dendritic patches was not significantly different from that at the soma (Fig. 1*b*). On average, the peak Na^+ current was 7.7 ± 1.5 pA (±s.e.m., $n = 10$) in somatic patches and 7.0 ± 0.9 pA ($n = 28$) in dendritic patches (excised 63–530 μm from the soma). This gives a somatic and dendritic Na^+ channel density of ~40 pS μm^{-2} (assuming a patch membrane area of ~1.5 μm^2; ref. 5). The finding that the apical dendrite of pyramidal neurons contains voltage-activated Na^+ channels agrees with previous work in both neocortical[6–8] and hippocampal pyramidal neurons[9–13]. In contrast to hippocampal pyramidal neurons,

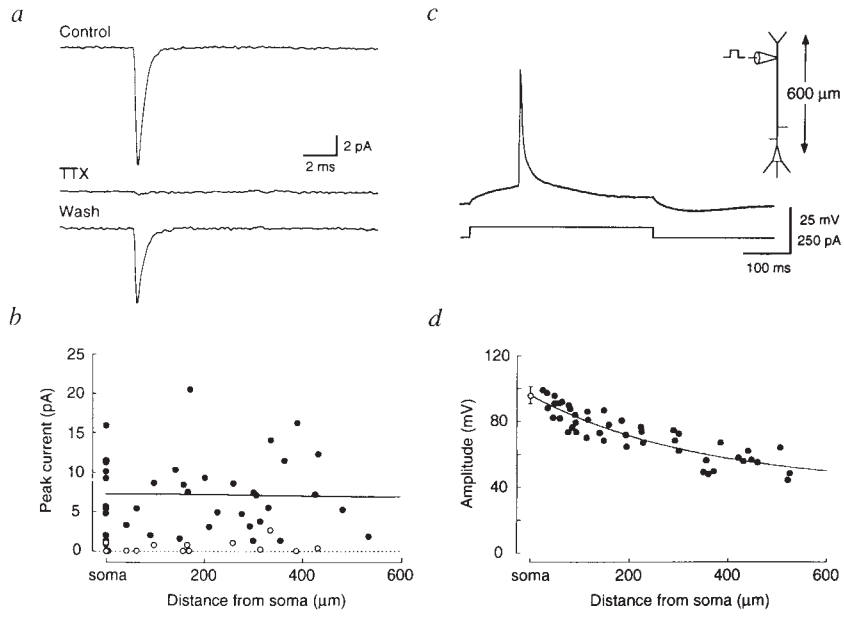

FIG. 1 Voltage-activated Na^+ currents and action potentials recorded from the apical dendrite of neocortical layer V pyramidal neurons. *a*, Rapidly inactivating inward current evoked in an outside-out dendritic patch excised from the apical dendrite of a layer V pyramidal neuron (439 μm from the soma). This current was blocked in the presence of 500 nM TTX. *b*, Plot of the peak inward current evoked in somatic ($n = 10$) and dendritic ($n = 28$) outside-out patches by depolarizations to the same test potential (filled symbols). The data have been fitted by a linear regression (solid line; regression coefficient is 0.028). When tested, this inward current was substantially reduced by 100 or 500 nM TTX (open symbols; $n = 14$). *c*, Dendritic action potential evoked at the resting membrane potential (−64 mV) by a 60 pA depolarizing current pulse during whole-cell recording from the apical dendrite of a layer V pyramidal neuron (440 μm from the soma). The inset in this and the following figures shows a schematic representation of the experimental recording arrangement. *d*, Plot of dendritic action potential amplitude (filled symbols) at different distances from the soma ($n = 39$; some cells were patched twice at different dendritic locations). The average amplitude (±s.d.) of somatic action potentials recorded in 16 of these cells is indicated (open symbol). The data have been arbitrarily fitted by a single exponential.

METHODS. Experiments were performed on 300-μm-thick parasagittal neocortical brain slices from 2-week-old Wistar rats. Slices were perfused with an oxygenated Ringer solution containing (in mM): 125 NaCl, 25 $NaHCO_3$, 25 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$ (pH 7.4) and, except when otherwise stated, experiments were performed at room temperature. Patch-clamp recordings were made from visually identified apical dendrites and somata of layer V pyramidal neurons using previously described techniques[29,30]. All outside-out patch-clamp recordings were made with a patch-clamp amplifier (EPC-7, List, Darmstadt, Germany) using 10-MΩ patch pipettes coated with Sylgard (184, Dow Corning) and filled with the following solution (in mM): 140 CsCl, 10 HEPES, 10 ethylene glycol tetraacetate (EGTA), 2 $MgCl_2$, 2 Na_2–ATP (pH 7.3). Rapidly inactivating inward currents were evoked by depolarizing steps to −10 mV from a holding potential of −90 mV and leak and capacitative currents were subtracted on-line (each trace is the average of 10 sweeps). Whole-cell recordings were made with a microelectrode amplifier (Axoclamp 2A, Axon Instruments, Foster City, US) using 10–15-MΩ patch pipettes filled with the following solution (in mM): 120 K-gluconate, 20 KCl, 10 HEPES, 10 EGTA, 2 $MgCl_2$, 2 Na_2–ATP (pH 7.3). Action potentials were evoked by depolarizing current pulses from the resting membrane potential and their amplitude was measured from a baseline set at threshold. The location of dendritic recordings was measured directly from the video monitor using an image processor (Argus 10, Hamamatsu, Hamamatsu City, Japan) and gives the distance from the centre of the soma to the dendritic recording site. Recordings were confirmed to be dendritic (outside-out patch recordings, $n = 4$; whole-cell recordings, $n = 6$) by identifying the recording site under fluorescence optics following filling of the neuron with the fluorescent dye Lucifer yellow (Sigma)[30].

however, where Na^+ channels are thought to be restricted to within 200 μm of the soma[12,13], neocortical pyramidal neurons express Na^+ channels along the entire length of the apical dendrite (Fig. 1*b*).

To investigate the possibility that dendritic Na^+ channels can initiate action potentials in the dendrites, as has been suggested in both neocortical[8,14–16] and hippocampal pyramidal cells[9,17–20], whole-cell voltage recordings were made from the apical dendrite of layer V pyramidal neurons. Action potentials could be evoked by depolarizing current pulses during recordings up to 525 μm from the soma (Fig. 1*c*). These action potentials, which were blocked by TTX (1 μM), were smaller and broader than somatic action potentials and their amplitude decreased with increasing distance from the soma (Fig. 1*d*). A similar decrease in dendritic action potential amplitude with increasing distance from the soma has also been observed in hippocampal pyramidal neurons[21].

One explanation for these findings is that dendritic action potentials represent an attenuated form of an action potential initiated close to the soma (where action potential amplitude is largest). To test this possibility, simultaneous whole-cell recordings were made from the soma and apical dendrite of the same cell. Action potentials evoked by dendritic current pulses (60–800 pA; 40–500 ms) were observed first at the soma (Fig. 2*a*; $n = 32$ at room temperature, dendritic recordings 60–525 μm from the soma; $n = 8$ at 35 °C, dendritic recordings 140–400 μm from the soma). That somatic and dendritic recordings were in fact made from the same cell, rather than electrically coupled cells[22], was demonstrated by simultaneously filling the same cell from the soma and the dendrite with different coloured fluorescent dyes (Fig. 2*b*; $n = 20$). Large-amplitude (up to 15 nA) dendritic current pulses of short duration (0.5 ms) were used in an attempt to initiate action potentials in the dendrites ($n = 5$ at 35 °C, dendritic recordings 140–350 μm from the soma). At threshold, action potentials evoked by short dendritic current pulses occurred first at the soma. As the amplitude of these

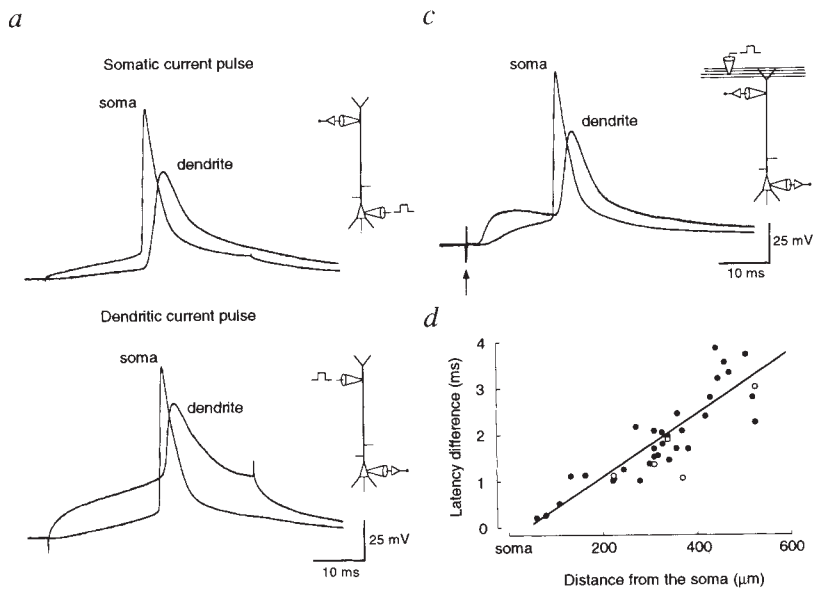

FIG. 2 Site of dendritic action potential initiation in neocortical layer V pyramidal neurons. *a*, Simultaneous whole-cell recordings were made from the soma and apical dendrite of the same layer V pyramidal neuron (dendritic recording 525 μm from the soma). Action potentials were evoked by depolarizing current pulse to either the soma (top; 150 pA) or dendrite (bottom; 300 pA). *b*, Simultaneous filling of the same layer V pyramidal neuron from the dendrite and the soma with different coloured fluorescent dyes: Cascade blue at the soma and Lucifer yellow in the dendrite (dendritic recording 190 μm from the soma). *c*, Action potentials initiated by distal synaptic stimulation in layer I (arrow) during simultaneous somatic and dendritic recording (dendritic recording 525 μm from the soma; same cell as in *a*). *d*, Plot of the difference in latency of the peak of dendritic and somatic action potentials during simultaneous somatic and dendritic recordings at different distances from the soma. Action potentials were initiated by either somatic current pulses (filled symbols; $n = 32$) or distal synaptic stimulation (open symbols; $n = 5$). The data have been fitted by a linear regression (regression coefficient is 0.88).

METHODS. Simultaneous whole-cell recordings were made from the soma and dendrite of the same visually identified layer V pyramidal cell using two identical microelectrode amplifiers (Axoclamp 2A). Action potentials were evoked by depolarizing current pulses to either the somatic or dendritic patch pipette. Recordings were only made from cells where it was possible to follow the apical dendrite from the cell soma to the site of dendritic recording. Cascade blue (Molecular Probes, Eugene, US) and Lucifer yellow (Sigma) were added directly to the intracellular solution at a concentration of 1 and 4 mg ml^{-1}, respectively. Both dyes can be excited by light with a wavelength of less than 410 nm and their fluorescence viewed simultaneously using an appropriate filter set (excitation BP 365/12, dichroic FT 395, emission LP 397; Zeiss). For synaptic stimulation, a third patch pipette (outer diameter 10–20 μm, filled with Ringer solution) was positioned under visual control in layer I and e.p.s.ps were evoked by brief voltage pulses (200 μs, up to 50 V).

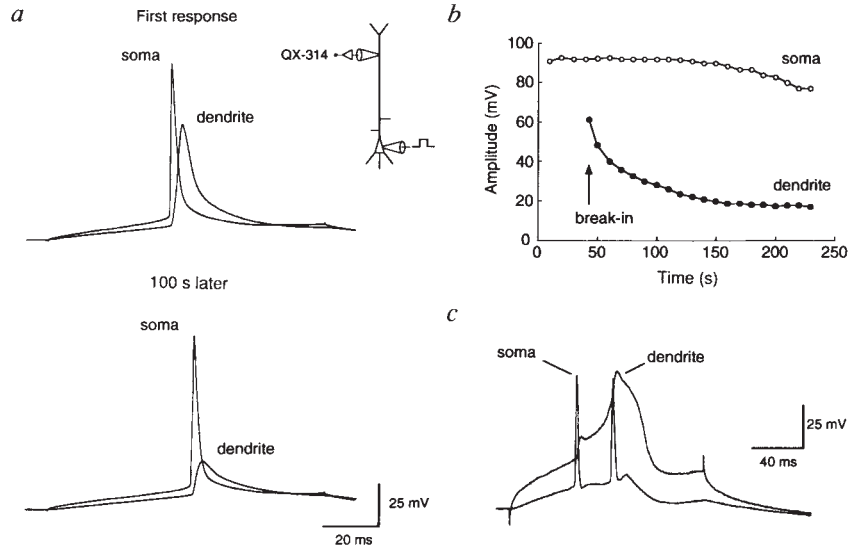

FIG. 3 Effect of the intracellular Na^+ channel blocker QX-314 on dendritic action potentials. *a*, Top, Somatic and dendritic action potentials, evoked by a depolarizing somatic current pulse (100 pA), during simultaneous somatic and dendritic recording immediately after obtaining a dendritic whole-cell recording (443 μm from the soma) with 10 mM QX-314 (Astra) in the dendritic patch pipette. Bottom, The same recording 100 s later shows that diffusion of QX-314 into the dendrite from the dendritic patch pipette substantially reduced the amplitude of the dendritic action potential with little effect on the action potential at the soma. *b*, The amplitude of the somatic (open symbols) and dendritic (filled symbols) action potentials for the experiment shown in *a* have been plotted against time. Break-in to obtain a dendritic whole-cell recording is indicated by the arrow. *c*, Dendritic Ca^{2+} spike evoked in isolation from the soma by a dendritic depolarizing current pulse (200 pA) during simultaneous somatic and dendritic recordings (dendritic recording 385 μm from the soma) with 1 mM QX-314 in the dendritic patch pipette.

dendritic current pulses was increased, however, the time between the peaks of the dendritic and somatic action potentials decreased, and on one occasion (260 μm from the soma, 5 nA current pulse) the dendritic action potential was observed to come before the somatic action potential. These results show that although action potential initiation usually occurs at or near the soma, it can shift into the dendrites during very strong dendritic depolarizations (to ~0 mV). To determine whether this can occur during synaptic stimulation, as has recently been suggested[8], action potentials were initiated by excitatory synaptic potentials (e.p.s.ps). Stimulation of excitatory synapses in layer I of the neocortex was used to depolarize the very distal regions of the dendritic tree. Action potentials initiated by these distal e.p.s.ps, which were larger and faster in the dendrite, were always observed to occur first at the soma (Fig. 2*c*; $n=5$ at room temperature; $n=8$ at 35 °C). Similar results were also obtained when e.p.s.ps were evoked by extracellular stimulation in other layers of the neocortex ($n=11$) or in the presence of bicuculline to block inhibition (5 μM). We conclude that, during synaptic stimulation of layer V pyramidal neurons, action potential initiation is at or near the soma.

To establish whether action potential initiation was somatic or axonal in origin, simultaneous recordings were obtained from the soma and axon of the same cell. Action potentials initiated by either somatic or axonal current pulses were always observed first in the axon ($n=4$; axonal recordings 10–35 μm from the soma). This provides direct evidence for the hypothesis that action potential initiation in neurons occurs in the axon, possibly at the axon initial segment, as suggested in the original studies on action potential initiation in motor neurons[1]. The finding that action potential initiation occurs first in the axon, rather than in the soma or dendrites, is presumably due to differences

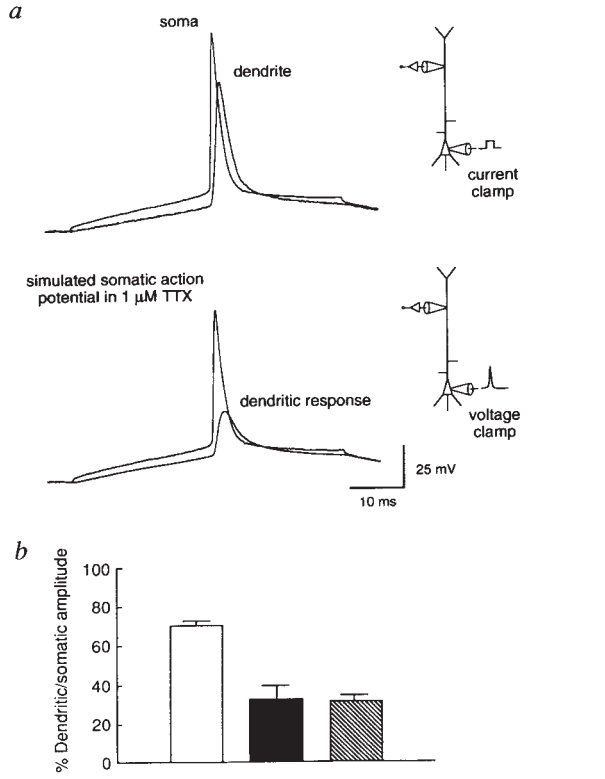

FIG. 4 Comparison of active and passive propagation of evoked action potentials and simulated action potential waveforms. *a*, Top, Somatic and dendritic action potentials evoked by a depolarizing somatic current pulse (200 pA) during simultaneous somatic and dendritic recording (dendritic recording 310 μm from the soma). Bottom, The dendritic response (at the same dendritic location) to a simulated somatic action potential waveform in the presence of TTX together with the actual voltage change at the soma during this simulated somatic action potential. *b*, Comparison of the average amplitude of dendritic action potentials (open column) and dendritic responses to inverted (hatched column) or upright (solid column) simulated somatic action potentials recorded at the same dendritic location (data expressed as a percentage of the response recorded at the soma ±s.e.m.; dendritic recordings 165–470 μm from the soma).
METHODS. Simultaneous whole-cell recordings were made using a patch-clamp amplifier (EPC-7) at the soma and a microelectrode amplifier (Axoclamp 2A) at the dendrite. Somatic and dendritic action potentials were evoked by a depolarizing somatic current pulse. The slice was then perfused with TTX (1 μM) and the previously recorded somatic action potential was used as a voltage-clamp command at the soma during somatic whole-cell voltage clamp. The dendritic response to this simulated action potential was then recorded at the same dendritic location. The actual voltage change at the soma during the simulated action potential was later recorded at the soma with a second somatic pipette. Somatic voltage-clamp patch pipettes (2–3 MΩ) were coated with Sylgard and series resistance (7–10 MΩ) was compensated by 70–90%. The action potential voltage command during somatic voltage clamp was scaled to one-tenth of its original size and inverted ($n=7$) or was the original size and upright in which case the experiments were performed in the presence of TTX ($n=5$). On three occasions both inverted and upright simulated action potentials were used in the same cells.

in the geometry, as well as to possible differences in the density, distribution and properties of voltage-activated channels in these structures[23].

The difference in latency of the peak of dendritic and somatic action potentials, evoked by somatic depolarization or synaptic stimulation, indicates a soma-to-dendrite action potential conduction velocity of ~0.15 m s^{-1} at room temperature (Fig. 2*d*), that is, the peak of an action potential takes 3–4 ms to propagate from the soma to the most distal apical dendrites. At 35 °C this conduction velocity was two to three times faster.

To investigate whether dendritic voltage-activated Na^+ channels aid the back-propagation of somatic action potentials into the dendrites, the internal Na^+ channel blocker QX-314 (ref. 24) was included in the dendritic patch pipette during simultaneous somatic and dendritic recording. Following establishment of dendritic recording, dendritic action potentials were observed to decrease progressively in amplitude before any change was observed in the amplitude or time course of the somatic action potential (Fig. 3*a*, *b*; $n=8$). This suggests that dendritic Na^+ channels are not involved in the initiation of somatic action potentials, but boost the amplitude of dendritic action potentials as they propagate back into the dendritic tree. In the presence of QX-314, dendritic Ca^{2+} spikes (blocked by 200 μM cadmium chloride) could be evoked in isolation from the soma (Fig. 3*c*), showing that, in addition to Na^+ channels, the dendrites of layer V pyramidal neurons also contain voltage-activated Ca^{2+} channels (see also ref. 7).

To compare the expected attenuation of dendritic action potentials in the presence and absence of dendritic Na^+ channels, voltage commands were applied at the soma to simulate a somatic action potential (in the presence of TTX or inverted) and the amount of attenuation of this simulated action potential waveform was compared with that of evoked action potentials. Somatic and dendritic action potentials were first recorded in response to a somatic current pulse (Fig. 4*a*; top). Following addition of 1 μM TTX, the same action potential as previously recorded at the soma was used as the voltage command during somatic voltage clamp and the response to this simulated action potential recorded at the same dendritic location (Fig. 4*a*; bottom). On average, for these experiments the amplitude of evoked dendritic action potentials was 70 ± 2.6% (±s.e.m., $n=9$) of somatic action potentials (during dendritic recordings 165–470 μm from the soma), whereas the dendritic response recorded at the same location to either a simulated upright action potential in TTX or an inverted action potential waveform (scaled to one-tenth the size of the somatic action potential) was 33 ± 6.7% ($n=5$) and 32 ± 3.3% ($n=7$) of that recorded at the soma (Fig. 4*b*). These results show unequivocally that somatic action potentials actively invade the dendrites of layer V pyramidal neurons via the activation of dendritic TTX-sensitive Na^+ channels.

Back-propagation of somatic action potentials provides neurons with a rapid retrograde signal that could modulate the computational properties of neurons in both the short and long term. For example, in the short term, dendritic action potentials may activate dendritic voltage-activated Ca^{2+} channels (Fig. 3*c*), leading to a rise in dendritic intracellular Ca^{2+} that could transiently shunt out parts of the dendritic tree by opening Ca^{2+}-dependent conductances. Sodium entry itself may have the same effect by opening dendritic Na^+-activated K^+ conductances. Active propagation of somatic action potentials into the dendritic tree may also be important for the induction of long-term changes in synaptic strength, either by direct activation of dendritic voltage-activated Ca^{2+} channels[13,25,28] or by boosting synaptic Ca^{2+} influx by briefly relieving the voltage-dependent Mg^{2+} block of dendritic *N*-methyl-D-aspartic acid receptor channels. □

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1. Eccles, J. C. *The Physiology of Synapses* (Springer, Berlin, 1964).
2. Rall, W. in *Handbook of Physiology. The Nervous System* (ed. Kandel, E. R.) 39–97 (Am. Physiol. Soc., Bethesda, Maryland, 1977).
3. Llinas, R. R. *Science* **242,** 1654–1664 (1988).
4. Adams, P. R. *Curr. Biol.* **2,** 625–627 (1992).
5. Sakmann, B. & Neher, E. in *Single-Channel Recording* (eds Sakmann, B. & Neher, E.) 37–51 (Plenum, New York, 1983).
6. Huguenard, J. R., Hamill, O. P. & Prince, D. A. *Proc. natn. Acad. Sci. U.S.A.* **86,** 2473–2477 (1989).
7. Amitai, Y., Friedman, A., Connors, B. W. & Gutnick, M. J. *Cerebral Cortex* **3,** 26–38 (1993).
8. Regehr, W., Kehoe, J., Ascher, P. & Armstrong, C. *Neuron* **11,** 145–151 (1993).
9. Wong, R. K. S., Prince, D. A. & Basbaum, A. I. *Proc. natn. Acad. Sci. U.S.A.* **76,** 986–990 (1979).
10. Benardo, L. S., Masukawa, L. M. & Prince, D. A. *J. Neurosci.* **2,** 1614–1622 (1982).
11. Miyakawa, H. & Kato, H. *Brain Res.* **399,** 303–309 (1986).
12. Turner, R. W., Meyers, D. E. R. & Barker, J. L. *J. Neurophysiol.* **62,** 1375–1387 (1989).
13. Jaffe, D. B. *et al. Nature* **357,** 244–246 (1992).
14. Purpura, D. P. in *The Neurosciences* (eds Quarton, G. C., Melnechuk, T. & Schmitt, F. O.) 372–393 (Rockefeller Univ. Press, New York, 1967).
15. Deschenes, M. *Expl Brain Res.* **43,** 304–308 (1981).
16. Pockberger, H. *Brain Res.* **539,** 181–190 (1991).
17. Spencer, W. A. & Kandel, E. R. *J. Neurophysiol.* **24,** 272–285 (1961).
18. Anderson, P. & Lomo, T. *Expl Brain Res.* **2,** 247–260 (1966).
19. Anderson, P., Storm, J. & Wheal, H. V. *J. Physiol., Lond.* **383,** 509–526 (1987).
20. Herreras, O. *J. Neurophysiol.* **64,** 1429–1441 (1990).
21. Turner, R. W., Meyers, D. E. R., Richardson, T. L. & Barker, J. L. *J. Neurosci.* **11,** 2270–2280 (1991).
22. Peinado, A., Yuste, R. & Katz, L. C. *Neuron* **10,** 103–114 (1993).
23. Moore, J. W., Stockbridge, N. & Westerfield, M. *J. Physiol., Lond.* **336,** 301–311 (1983).
24. Strichartz, G. R. *J. gen. Physiol.* **62,** 37–57 (1973).
25. Artola, A., Bröcher, S. & Singer, W. *Nature* **347,** 69–72 (1990).
26. Grover, L. M. & Teyler, T. J. *Nature* **347,** 477–479 (1990).
27. Jaffe, D. & Johnston, D. *J. Neurophysiol.* **64,** 948–960 (1990).
28. Komatsu, Y. & Iwakiri, M. *J. Neurophysiol.* **67,** 401–410 (1992).
29. Dodt, H.-U. & Zieglgänsberger, W. *Brain Res.* **537,** 333–336 (1990).
30. Stuart, G. J., Dodt, H.-U. & Sakmann, B. *Pflügers Arch. ges. Physiol.* **423,** 511–518 (1993).

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Three types of Ca^{2+} channel trigger secretion with different efficacies in chromaffin cells

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To determine whether the different types of Ca^{2+} channels present in the same secretory cell contribute equally to secretion, we used chromaffin cells to analyse the coupling between three distinct types of Ca^{2+} channel[1] and exocytosis[2,3]. These are ω-conotoxin-GVIA-sensitive[4,5] N-type channels, ω-agatoxin-IVA-sensitive P-type Ca^{2+} channels[6] and dihydropyridine-sensitive facilitation Ca^{2+} channels, which are normally quiescent but are activated by depolarizing pre-pulses, repetitive depolarizations to physiological potentials[7,8], or agents that raise cyclic AMP[9]. We have simultaneously monitored changes in capacitance as an assay of catecholamine secretion[10], and Ca^{2+} currents. Although all three types of Ca^{2+} channel trigger secretion individually, facilitation channels produce much greater secretion for a given size of Ca^{2+} current, indicating that they are coupled more efficiently to exocytosis. These results indicate that facilitation Ca^{2+} channels may be physically nearer vesicle release sites. They also show that low efficiency P- and N-type channels could trigger mild release and that high-efficiency facilitation channels may underlie the massive catecholamine release that occurs during the 'fight or flight' response.