show a decline in acuity in the presence of multiple sources²³, as do earphone studies with stimuli containing 'interfering' ITDs^{24–26}. Our results suggest that the broadly tuned nature of the population code is the neural basis for this fundamental limitation. We suggest that to the extent we are aware of the locations of numerous, simultaneous sources, this 'auditory image' must be constructed on the basis of sequential determinations from different sources at different times. □

Methods

Animals ($n = 31$) were surgically prepared under ketamine/xylazine anaesthesia by attaching a bar to the skull with screws and dental cement. In a second surgery, a small hole was drilled in the skull overlying the cortex and filled with removable elastomer. Recordings were made without anaesthetic. The animals were restrained by securing the body in a close-fitting bag and clamping the head bar. The elastomer was removed, local anaesthetic was applied to the dura, and the electrode was directed towards an auditory centre. Daily recordings lasting ~2 hours were made over a period of up to several months. Usually only a single nucleus was studied. Recordings consisted of single neurons or clusters of a few neurons. Responses were obtained to 'binaural-beat' stimuli, which were pure tones (3.1 to 5.1 s long and repeated 2–4 times at a constant sound pressure level, usually near 70 dB) to each ear that differed in frequency by 1 Hz. This frequency difference created a cyclically varying ITD with a period of 1 s. Period histograms of the responses were created based on the 1-Hz binaural beat frequency; these yield the response as a function of interaural time difference. In our unanaesthetized preparation there was little if any difference in ITD tuning widths to static or dynamic (binaural beat) stimuli. Curves (Fig. 1a) were smoothed by counting spikes in 10 bins per cycle of the stimulating frequency. For all calculations used in later figures, 25 bins per cycle were used. The initial 100 ms was omitted to avoid onset effects. A full description of recording and stimulus conditions is provided elsewhere^{27,28}.

Received 18 February; accepted 4 June 1997.

- Mills, A. W. On the minimum audible angle. *J. Acoust. Soc. Am.* **65**, 991–1000 (1958).
- Kuwada, S., Batra, R. & Fitzpatrick, D. C. in *Binaural and Spatial Hearing in Real and Virtual Environments* (eds Gilkey, R. H. & Anderson, T. R.) 399–426 (Erlbaum, Mahwah, NJ, 1997).
- Brugge, J. F., Reale, R. A. & Hind, J. E. in *Binaural and Spatial Hearing in Real and Virtual Environments* (eds Gilkey, R. H. & Anderson, T. R.) 447–474 (Erlbaum, Mahwah, NJ, 1997).
- Blauert, J. *Spatial Hearing: The Psychophysics of Human Sound Localization* (MIT Press, Cambridge, MA, 1982).
- Wightman, F. L. & Kistler, D. J. The dominant role of low frequency interaural time differences in sound localization. *J. Acoust. Soc. Am.* **91**, 1648–1661 (1992).
- Yin, T. C. T. & Kuwada, S. Binaural interaction in low-frequency neurons in inferior colliculus of the cat. III. Effects of changing frequency. *J. Neurophysiol.* **50**, 1020–1042 (1983).
- Li, L. & Kelly, J. B. Inhibitory influence of the dorsal nucleus of the lateral lemniscus on binaural responses in the rat's inferior colliculus. *J. Neurosci.* **12**, 4530–4539 (1992).
- Stern, R. M. & Trahiotis, C. in *Auditory Physiology and Perception* (eds Cazals, Y., Horner, K. & Demany, J.) 547–554 (Pergamon, Oxford, 1992).
- Georgopoulos, A. P., Schwartz, A. B. & Kettner, R. E. Neuronal population coding of movement direction. *Science* **233**, 1416–1419 (1986).
- Georgopoulos, A. P., Kettner, R. E. & Schwartz, A. B. Primate motor cortex and free arm movements to visual targets in three-dimensional space. II. Coding of the direction of movement by a neuronal population. *J. Neurosci.* **8**, 2928–2937 (1988).
- Stern, R. M. & Colburn, H. S. Theory of binaural interaction based on auditory-nerve data IV. A model for subjective lateral position. *J. Acoust. Soc. Am.* **64**, 127–140 (1978).
- Green, D. M. & Swets, J. A. in *Signal Detection Theory and Psychophysics* 1–455 (Wiley, New York, 1966).
- Hinton, G. E., McClelland, J. L. & Rumelhart, D. E. in *Parallel Distributed Processing* Vol. 1 (eds Rumelhart, D. E. & McClelland, J. L.) 77–109 (MIT Press, Cambridge, MA, 1986).
- Salinas, E. & Abbot, L. F. Vector reconstruction from firing rates. *J. Comp. Neurosci.* **1**, 89–97 (1994).
- Hefner, H. & Masterton, B. Hearing in Glires domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. *J. Acoust. Soc. Am.* **68**, 1584–1599 (1980).
- Haft, E. R. & DeMaio, J. Difference thresholds for interaural delay. *J. Acoust. Soc. Am.* **57**, 181–187 (1975).
- Hubel, D. H. & Wiesel, T. N. Receptive fields. Binocular interactions and functional architecture in the cat's visual cortex. *J. Physiol. (Lond.)* **160**, 106–154 (1962).
- Capuano, U. & McIlwain, J. T. Reciprocity of receptive field images and point images in the superior colliculus of the cat. *J. Comp. Neurosci.* **196**, 13–23 (1981).
- Rummelhart, D. E., Smolensky, P., Hinton, G. E. & McClelland, J. L. in *Parallel Distributed Processing* Vol. II (eds McClelland, J. L. & Rumelhart, D. E.) 77–109 (MIT Press, Cambridge, MA, 1986).
- Van Gisbergen, J. A. M., Van Opstal, A. J. & Tax, A. A. Collicular ensemble coding of saccades based on vector summation. *Neuroscience* **21**, 541–555 (1987).
- Lee, C., Rohrer, W. H. & Sparks, D. L. Population coding of saccadic eye movements by neurons in the superior colliculus. *Nature* **332**, 357–360 (1988).
- Theunissen, F. & Miller, J. P. Representation of sensory information in the cricket cercal sensory systems. II. Information theoretic calculation of system accuracy and optimal tuning curve widths of four primary interneurons. *J. Neurophysiol.* **66**, 1690–1703 (1991).
- Perrott, D. R. Concurrent minimum audible angle: a re-examination of the concept of auditory spatial acuity. *J. Acoust. Soc. Am.* **75**, 1201–1206 (1984).
- Trahiotis, C. & Bernstein, C. R. Detectability of interaural delays over select spectral regions: Effects of flanking noise. *J. Acoust. Soc. Am.* **87**, 810–913 (1990).

- Dye, R. H. The combination of interaural information across frequencies: Lateralization of the basis of interaural delay. *J. Acoust. Soc. Am.* **88**, 2159–2170 (1990).
- Heller, L. M. & Trahiotis, C. Extents of laterality and binaural interference effects. *J. Acoust. Soc. Am.* **99**, 3632–3637 (1996).
- Kuwada, S., Stanford, T. R. & Batra, R. Interaural phase sensitive units in the inferior colliculus of the unanesthetized rabbit. Effects of changing frequency. *J. Neurophysiol.* **57**, 1338–1360 (1987).
- Batra, R., Kuwada, S. & Stanford, T. R. Temporal coding of envelopes and their interaural delays in the inferior colliculus of the unanesthetized rabbit. *J. Neurophysiol.* **61**, 257–268 (1989).
- Batra, R., Kuwada, S. & Fitzpatrick, D. C. Sensitivity to interaural temporal disparities of low and high frequency neurons in the superior olivary complex. I. Heterogeneity of responses. *J. Neurophysiol.* (in the press).
- Stanford, T. R., Kuwada, S. & Batra, R. A comparison of the interaural time sensitivity of neurons in the inferior colliculus and thalamus of the unanesthetized rabbit. *J. Neurosci.* **12**, 3200–3216 (1992).

Acknowledgements. We thank C. Trahiotis, L. Bernstein and L. Abbott for discussion, L. M. Seman for technical assistance, and T. Ju and R. Manfredi for computer programming. This work was supported by a grant from the Center for Deafness and Communicative Disorders, NIH, USA.

Correspondence and requests for materials should be addressed to D.C.F. (e-mail: dcf@neuron.uchc.edu).

Loose-patch recordings of single quanta at individual hippocampal synapses

Lia Forti, Mario Bossi, Andrea Bergamaschi, Antonello Villa & Antonio Malgaroli

Department of Biological and Technological Research, DIBIT, Scientific Institute San Raffaele, and Cellular and Molecular Pharmacology Center, CNR, Department of Pharmacology, University of Milano, Via Olgettina 58, 20123 Milano, Italy

Synapses in the central nervous system are typically studied by recording electrical responses from the cell body of the post-synaptic cell. Because neurons are normally connected by multiple synaptic contacts, these postsynaptic responses reflect the combined activity of many thousands of synapses, and it remains unclear to what extent the properties of individual synapses can be deduced from the population response^{1–5}. We have therefore developed a method for recording the activity of individual hippocampal synapses. By capturing an isolated presynaptic bouton inside a loose-patch pipette and recording from the associated patch of postsynaptic membrane, we were able to detect miniature excitatory postsynaptic currents ('minis') arising from spontaneous vesicle exocytosis at a single synaptic site, and to compare these with minis recorded simultaneously from the cell body. The average peak conductance at a single synapse was about 900 pS, corresponding roughly to the opening of 90 AMPA-type glutamate-receptor channels. The variability in this conductance was about 30%, matching the value reported for the neuromuscular junction⁶. Given that our synapses displayed single postsynaptic densities (PSDs), this variability is larger than would be predicted from the random opening of receptor channels, suggesting that they are not saturated by the content of a single vesicle. Therefore the response to a quantum of neurotransmitter at these synapses is not limited by the number of available postsynaptic receptors.

Individual CA3–CA1 hippocampal boutons, visualized with the fluorescent dye FM1-43 (refs 7, 8), were enclosed inside patch pipettes and loose-seals⁹ were gently formed with the dendritic membrane underneath (Fig. 1a, b). In this configuration, quantal signals from synapses positioned outside the pipette are not visible above background noise (see Methods). Thus this approach provides a simple way of looking at currents from single synapses in isolation. For synaptic loose-seal recordings, we selected isolated chains of fluorescent boutons, belonging to putative single axons, above large proximal dendrites (~0–70 µm from the end of the soma) (Fig. 1a). The morphology of these contacts was analysed by serial electron microscopy (EM) of proximal dendrites. At these

locations, synapses were found to be discrete entities, with elliptically shaped varicosities (longitudinal diameter, $1.67 \pm 0.60 \mu\text{m}$; $n = 37$ synapses) filled with small synaptic vesicles (SSVs) (158.4 ± 91.7 SSVs; $n = 37$ serially reconstructed synapses), 12% of which were docked to the active region (18.6 ± 11.4 docked SSVs/synapse) (Fig. 1d). In 97% of cases ($n = 36/37$), presynaptic varicosities were apposed to individual postsynaptic densities (PSDs) having an average diameter of 441.6 ± 77.8 nm (Fig. 1c, d). Most of these synapses were composed of well differentiated spines and no glial layers were wrapped around the synaptic junctions (Fig. 1c). The average intersynaptic distance along individual axon collaterals was $4.42 \pm 1.91 \mu\text{m}$ ($n = 95$) (Fig. 1e), in agreement with measurements obtained by fluorescence confocal microscopy (5.12 ± 2.69 ; $n = 256$). Based on these morphological results, we conclude that an individual synapse can be contained inside a recording pipette with $\sim 2\text{-}\mu\text{m}$ tip, whereas the probability to accommodate two intact boutons inside is very low ($\sim 1\%$) (Fig. 1e).

Using this recording configuration (tetrodotoxin (TTX), AP5 (D-2-amino-5-phosphonovaleric acid) and picrotoxin always

present), we could detect AMPA-receptor-mediated miniature currents, always with inward polarity, in 18% of the synaptic sites investigated ($n = 22/125$) (Fig. 2a). At an individual site, minis exhibited variable amplitudes (mean peak amplitude = 53.6 ± 14.7 pA; $n = 11$) (Fig. 2a, b) but a remarkably similar time course (Fig. 2b). The time course of the average synaptic current was characterized by a fast rise ($t_{20-80\%} = 158 \pm 46 \mu\text{s}$; $n = 9$), followed by a slower decay, best described in most experiments by the sum of two exponential components (Fig. 2c) (time constants: $\tau_{\text{fast}} = 0.61 \pm 0.26$ ms; $\tau_{\text{slow}} = 2.55 \pm 1.19$ ms; peak amplitude ratio: $a_{\text{slow}}/a_{\text{fast}} = 0.33 \pm 0.23$; $n = 6$; $P < 0.025$). The presence of a biexponential decay time course could reflect either a complex glutamate kinetics in the synaptic cleft or different functional and/or molecular isoforms of AMPA channels at these synapses. To characterize these signals further, we carried out experiments in the presence of somatic voltage clamp. A somatic whole-cell (WC) recording was established before lowering the loose-patch pipette to enclose the selected fluorescent bouton and, as shown in Fig. 3a, when spontaneous events were detected at a synaptic site, synchronous minis were

Figure 1 Loose-patch synaptic recordings. **a**, A fluorescence image of FM1-43 labelled synaptic boutons superimposed to the transmitted light view of a hippocampal neuron. A fluorescent synapse on a proximal dendrite is enclosed inside a patch pipette. Scale bar, $10 \mu\text{m}$. **b**, The recording configuration: the recording pipette forms a loose seal onto the postsynaptic membrane. **c**, Electron micrograph of an axon making two consecutive synapses along a proximal dendrite. Scale bar, $1 \mu\text{m}$. During loose-seal formation, the thin axon is presumably compressed between the pipette and the dendritic membrane. **d**, Numbers of total small synaptic vesicles (SSVs), docked SSVs/active region and longitudinal diameter of PSD from serially reconstructed synapses ($n = 37$ synapses; mean $\pm$ s.e.m.). **e**, Histogram of intersynaptic distances between centres of PSDs along individual axons as in **c** ($n = 95$). The compound probability for two intact boutons (longitudinal diameter, $1.67 \pm 0.60 \mu\text{m}$; $n = 37$) to fit inside the recording pipette (tip diameter (ϕ), $\sim 2 \mu\text{m}$) was $\sim 1\%$.

Figure 2 Miniature currents from single CA3-CA1 hippocampal synapses. **a**, Current traces from an exemplar single-synapse recording. Scale: 60 pA , 20 ms ($1 + R_{\text{pipette}}/R_{\text{seal}} = 1.33$; 2 kHz low-pass filtering; experiment LP008). **b**, Superimposition of 5 minis from the same experiment. Scale: 20 pA , 3 ms . Inset, the same events are shown after peak amplitude normalization to illustrate their almost identical time course. **c**, Average synaptic current from the same recording ($n = 14$ minis), with superimposed biexponential fit (solid line) to the decay phase ($a_{\text{slow}}/a_{\text{fast}} = 0.46$). Rise time ($t_{20-80\%}$) was $170 \mu\text{s}$. Scale: 12 pA , 3 ms . Inset, symbols plot the decay time constants (τ_{slow} and τ_{fast}) from 6/9 experiments where the decay timecourse was best described by the sum of two exponential components.

also present in the whole-cell trace ($n = 11/27$ experiments). In these experiments, focally recorded minis did not differ in amplitude and time course from those acquired in unclamped conditions. The amplitudes of single-synapse events detected simultaneously at the site of origin and at the soma were highly correlated, suggesting a linear propagation of minis along the dendritic cable (correlation coefficient, $r = 0.8 \pm 0.07$; $P < 10^{-4}$, $n = 4$ experiments) (Fig. 3b, inset). Across different experiments, a variable degree of amplitude attenuation (range, 0.29–0.96)

and slowing down of the time course was found (Fig. 3a, b), with no clear relation with either distance of the recorded sites from the soma or series resistance values ($R_s = 10$ – $20 \text{ M}\Omega$). Because, at individual synapses, quantal release events were consistently clustered in time (high- Ca^{2+} solution present in loose pipettes), in double recording experiments this produced correlated frequency fluctuations in quantal rate in both whole-cell and loose-patch traces (Fig. 3c). From these experiments, a direct estimate of the real synaptic conductance was obtained,

Figure 3 Simultaneous synaptic and somatic-wc recordings. **a**, Current traces from a loose-synaptic (lp; upper traces; $1 + R_{\text{pipette}}/R_{\text{seal}} = 1.53$) and somatic (WC; lower traces; holding potential, -55 mV) pair recording. Notice how minis originating from the synapse enclosed in the loose-patch pipette appear synchronously in both traces, whereas minis from other synapses are exclusively seen with the WC electrode (3 kHz low pass filtering; experiment LP064). **b**, Comparison of the average synaptic current from 130 paired minis recorded at the synaptic site (thick line; scaled by 1.53) or at the soma (thin line) (experiment LP064). Inset, correlation between amplitudes of single-synapse minis measured at the synaptic site (I_{lp}) and at the soma (I_{wc}) within the same experiment. The dotted line is a linear regression to all points ($R = 0.81$, slope = 0.29 , $P < 10^{-4}$, $R_s = 16 \text{ M}\Omega$). The linear relation between I_{lp} and I_{wc} suggests linear propagation of minis along the dendritic cable. **c**, Mini frequency time plots from a double-recording experiment. R_s for the WC recording is plotted below ($R = 11 \text{ M}\Omega$). Notice that the formation of the loose-seal (arrow) did not induce any visible perturbation in the WC recordings. Also, correlated frequency fluctuations are present in both plots, reflecting clustering of quanta at the recorded synapse (single, lp). Additional clusters appear in the somatic trace (population, WC) at $t \sim 30$ and ~ 340 s (experiment LP053).

Figure 4 Quantal variability at single hippocampal synapses. **a**, Pooled values of amplitude CVs for population (Pop) and single-bouton minis (Single) (n , 7, 4, 4 and 7 experiments; mean $\pm$ s.d.). Minis were collected either focally (lp) or at the soma (WC) (Dual rec., simultaneous WC and synaptic recordings). CVs of population and single-bouton minis are significantly different ($P < 10^{-3}$, independent t -test). CV values for single-synapse minis, obtained under the various recording conditions, do not differ significantly from each other ($P > 0.05$, paired and independent t -tests). Symbols on the right plot all individual CV values ($n = 11$). The small horizontal line is their mean value. **b**, Single-synapse (top traces) and population minis (bottom traces) recorded at the soma. Note that single-synapse minis display a larger waveform similarity than do population events (experiment LP053). **c**, Amplitude histograms of 179 single-synapse minis measured focally (lp) (mean, 45.8 pA ; CV = 0.33 ; $\sigma_{\text{noise}} = 3.4 \text{ pA}$, 3 kHz low pass filtering) or after propagation to the somatic pipette (WC) (mean, 13.7 pA ; CV = 0.34 ; $\sigma_{\text{noise}} = 1.9 \text{ pA}$; 3 kHz filtering). The thin line is a gaussian function superimposed on the lp histogram (experiment LP064). Inset, cumulative plots of the same set of data superimposed to the amplitude distribution of population WC minis, recorded during the same experimental epoch (population) ($n = 685$; mean, 25.5 pA , CV = 0.68). Distributions were normalized to median values (Norm. Ampl.). Note the perfect overlap of the two single-synapse distributions (KS = 0.9) and the significant divergence from the distribution of population quanta (KS $< 10^{-3}$), which shows a pronounced positive skew (skew $_{wc=lp}$ = 1.94 and skew $_{wc=population}$ = 19.05 times the values expected for normally distributed data).

with a mean value of 886 ± 180 pS (range, 650–1,050 pS; holding potential (h.p.) = $-50/-60$ mV; $n = 4$). As the elementary conductance for AMPA channels in CA3–CA1 neurons is ~ 10 pS (ref. 10), this would translate into ~ 90 AMPA channels open at the peak of a mEPSC. This figure could be a slight underestimate, because in higher divalent concentrations a small reduction in AMPA conductance is expected¹¹.

When miniatures are collected from the somata of central neurons, a large quantal variability is consistently found^{3–5,12–14}, with broad and skewed amplitude distributions. This contrasts with the low unitary variability obtained from deconvolution of evoked distributions^{11,12,15–18} and many explanations for this discrepancy have been proposed^{1,2,4,11,19–21}. With synaptic loose-seal recordings, we found that the average degree of amplitude variability (coefficient of variation, CV = s.d./mean) was $28.4 \pm 7.2\%$ ($n = 11$), a value more than twofold lower than that found with standard somatic whole-cell recordings, in which the combined activity of many synapses was sampled ($72.4 \pm 15.5\%$; $n = 7$) (Fig. 4a). A large dilation of single-synapse CVs through synaptic fatigue processes, such as AMPA-receptor desensitization, is unlikely because in most experiments no correlation between mini amplitude and inter-event intervals was found ($P > 0.1$, $n = 8/11$; see Methods). In double-recording experiments (somatic whole cell and synaptic loose seal), we could directly compare properties of minis generated at individual synapses with those of events arising at the remaining synapses (population minis). In these experiments, single synapse minis displayed CVs significantly lower than those of population minis, both measured from somatic whole-cell traces ($CV_{wc-single} = 33 \pm 5.9\%$; $CV_{wc-population} = 53 \pm 11\%$; $P < 10^{-3}$, paired *t*-test; $n = 4$) (Fig. 4a). In addition, as shown in Fig. 4b, the time course of single-synapse minis was highly conserved, in contrast with the largely scattered waveforms of population minis, and this produced a significant difference in the respective waveform cross-correlation coefficients ($P < 0.05$; $n = 4$). When amplitude distributions for single-synapse minis were constructed, these displayed nearly gaussian shapes (Fig. 4c). Current propagation to the soma shifted amplitudes of single-synapse events towards lower values (Fig. 4c: WC versus Ip) but did not alter the shape of the underlying distributions; this was better visualized by superimposing the two normalized cumulative distributions (Fig. 4c, inset) ($KS > 0.9$, where *KS* is the Kolmogorov–Smirnov test on cumulative averages; $n = 4$ experiments). In the same set of experiments, a sharp divergence from the shape of amplitude distributions for population minis was revealed ($KS < 0.005$; as above), the latter exhibiting the characteristic pronounced skewness (Fig. 4c, inset) ($skew_{wc-single} = 2.1 \pm 1.7$ and $skew_{wc-population} = 15.2 \pm 4.8$ times the values expected for normally distributed data; $n = 4$ experiments). The small quantal variability observed at individual synapses cannot be attributed to the high- Ca^{2+} solution in loose-seal electrodes because in two synaptic recordings in which loose pipettes were filled with control Tyrode and some minis could be detected ($n = 2/37$), quantal variability matched values obtained in high- Ca^{2+} ($CV = 0.32$ and 0.43 ; $n = 21$ and 12 events). In addition, bath application of the same high- Ca^{2+} solution while recording minis from the cell soma (single-pipette whole-cell experiments) did not reduce but further increased the skewness of amplitude distributions ($skew_{wc-control} = 19.2 \pm 9.3$ and $skew_{wc-highCa2+} = 27.8 \pm 11.1$, as above), with a slight reduction in mini size ($12 \pm 21\%$), and in amplitude variability (17% ; $CV_{wc-control} = 81.5 \pm 10.6\%$; $CV_{wc-highCa2+} = 67.6 \pm 15.2\%$), which remained significantly higher than at single synapses ($P < 10^{-3}$). Taken together, these results indicate that the large variability of population minis (as detected by somatic recordings), rather than reflecting an intrinsic peculiarity of spontaneous transmission at one synapse, arises from the summed behaviour of many synaptic terminals. As in our system synapses on a given postsynaptic neuron display similar mean amplitudes⁴, then much of the observed

variability might originate from variable electronic filtering of synaptic currents injected at variable distances from the soma^{22,23}. Single-synapse CVs always in excess of the value expected from stochastic channel gating ($< 10\%$), in a condition where synapses display unitary PSDs, strongly suggests an incomplete occupancy of AMPA receptors after the release of one quantum^{24–27}.

In conclusion, our findings show that it is possible to record from a single central synapse and that its quantal properties can be accurately estimated. Our results show that quantal variability at individual glutamatergic synapses is far shorter than that seen in population recordings. Unimodal amplitude distributions and lack of receptor saturation suggest that minis, in this system, are likely to be unquantal events corresponding to the opening of ~ 90 AMPA channels. From this value, a rough estimate of 150–180 total available channels at an individual synaptic site can be obtained, assuming an open probability for AMPA channels of 0.5–0.6 at the peak of the synaptic event¹⁰. The high number of recordings from FM1–43 labelled synapses, where minis were not present (82%), besides the intrinsic technical difficulties could reflect lack of functional postsynaptic AMPA receptors, from enclosure inside loose pipettes of either GABAergic inhibitory boutons, or purely NMDA synapses^{28–30}. □

Methods

Synaptic loose-patch recordings. Postnatal CA3–CA1 hippocampal cultures were prepared as previously described¹⁴. Neurons (6–21 days old) were superfused ($22–25^{\circ}C$) with a solution containing (in mM): NaCl 119, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, HEPES 25, glucose 30; and picrotoxin, 100 μM (Sigma), APV 25–100 μM (Tocris), TTX 0.5 μM (Latoxan), adjusted to 305 mOsm and pH 7.4 (control Tyrode solution). Synapses were labelled for 1–2 min with 10 μM FM1–43 (Molecular Probes) dissolved in modified Tyrode solutions (either 90 mM KCl or, in most experiments, 5 mM $CaCl_2$ /no added $MgCl_2$; 25 μM CNQX, Tocris, 0.5–1 mM kynurenate, Tocris). The recording pipette (tip diameter ~ 2 μm ; resistance, $R_{pipette}$, 1–2 M Ω), filled with a high- Ca^{2+} Tyrode solution ($CaCl_2$ 5 mM, no added $MgCl_2$; picrotoxin 100 μM , APV 25–100 μM , TTX 0.5 μM), was connected to a patch-clamp amplifier (Axopatch 200A; Axon) in voltage-clamp mode (VC) and held at the zero-current potential. Pipettes were lowered to enclose selected boutons and loose seals (seal resistance, R_{seal} , 2–11 M Ω) were obtained without suction. During recordings, 1-mV potential steps were applied every 2 s to monitor the stability of seal resistance. Experimental epochs accepted for amplitude analysis did not display seal resistance variation in excess of 5% of mean value and had less than 1 mV drift in ($V_{pipette} - V_{bath}$). With R_{seal} values of about two orders of magnitude larger than bath resistance, less than $\sim 1\%$ of ‘contaminating’ currents from synapses located outside the pipette would flow to ground through the electrode (below detection threshold, for signals up to a few hundred pA). The large frequency fluctuations in quantal rate in loose-seal recordings were induced by the high Ca^{2+} in the recording pipette as they were not seen in 37/37 loose-patch experiments with pipettes filled with control Tyrode (thus also excluding disruption of terminals with loose pipettes). All current traces were stored on a PCM-video recorder.

Somatic whole-cell recordings. Somatic voltage clamp recordings were obtained either in whole-cell or perforated patch configuration (amphotericin B from Sigma at 0.25 $\mu g ml^{-1}$) (EPC-7, List, or Axopatch 1D, Axon). Patch electrodes (2–5 M Ω) contained (mM): caesium gluconate 110, $MgCl_2$ 5, NaCl 10, EGTA or BAPTA 0.6–10, ATP 2, GTP 0.2, HEPES 49, adjusted to pH 7.2 and 290 mOsm. Input resistance (0.1–1.2 G Ω) and series resistance (R_s ; 5–20 M Ω) were continuously monitored for constancy and experiments were rejected if they varied by more than 15%. All minis were suppressed by application of CNQX (10 μM ; see also ref. 14).

Data analysis. Data were low-pass-filtered at 2–5 kHz and digitized at 10–70 kHz. Minis were detected semi-automatically using two threshold-crossing criteria on the current signal and on its first derivative (thresholds: 3 times background s.d. Computer simulations estimated the undetected events to be less than 3% for signal-to-noise ratios (*S/N*) > 4 and less than 20% for *S/N* between 3 and 4. In loose-patch recordings (background s.d. range: 2.8–5.4 pA) mini amplitude values used for mean amplitude estimate and histogram

display were corrected for current loss through the seal⁹ (correction factor $C = 1 + R_{\text{pipette}}/R_{\text{seal}}$; range 1.2–1.8). In all analysed regions, measured mini amplitude was stationary (no correlation between amplitude and time; $P > 0.1$). Mini amplitude CVs were computed from 18–179 (mean, 101 ± 54) events as $\sqrt{(\text{total variance of measured amplitudes} - \text{background variance})/\text{mean}}$; the estimated contribution of variance arising from current loss through the loose seal was negligible. No correlation between mini amplitudes and inter-mini intervals was found in 8/11 experiments ($P > 0.1$). In 3 experiments, a positive correlation was present and disappeared after removal of those few events with intervals below 15–20 ms, without any significant change in CV estimates. For averages of synaptic currents, events were aligned to $t_{1/2}$ of rise. The kinetics of focally recorded minis were slightly faster than for currents obtained with fast glutamate application on outside-out patches¹⁰, suggesting either a difference in AMPA receptors properties (extra-synaptic versus synaptic, slices versus cultures) or different glutamate time courses in the two conditions. Mini decays were fitted, using a nonlinear least-squares algorithm, with the sum of 1–2 exponential components (minimum number chosen by the likelihood ratio test). For analysis of waveform variability in double-recording experiments, couples of mini waveforms from the whole-cell trace, aligned to their starting point, were cross-correlated over 8 ms. Cross-correlation coefficients (ρ) for consecutive single synapse minis were compared with ρ values for consecutive population minis from the same trace using a Fisher z -transform and independent t -test ($P < 0.05$ considered as significant). Values throughout the text are means $\pm$ s.d. unless specified.

Electron and fluorescence microscopy. For standard EM, cell monolayers were washed with control Tyrode solution, fixed with 2% glutaraldehyde/4% paraformaldehyde in 0.1 M cacodylate buffer (45 min, 24 °C) and post-fixed with 2% OsO₄. After dehydration in ethanol and overnight infiltration (75% Epon-812, 25% ethanol), samples were embedded in Epon. Ultrathin serial sections (~ 60 nm; ≥ 4 sections per synapse; mean, 5.5) were doubly stained with uranyl acetate and lead citrate and examined with a Hitachi H-7000 microscope. Fluorescence measurements of intersynaptic distances were obtained after labelling with a polyclonal anti-synaptotagmin antibody using a confocal microscope (Biorad MRC1024). The fluorescence picture in Fig. 1a was obtained with an SIT camera (Hamamatsu C5985) attached to the recording microscope.

Received 23 January; accepted 6 June 1997.

- Jack, J., Larkman, A. U., Major, G. & Stratford, K. J. in *Molecular and Cellular Mechanisms of Neurotransmitter Release* (eds Stjärne, L. et al.) 275–299 (Raven, New York, 1994).
- Edwards, F. A. Anatomy and electrophysiology of fast central synapses lead to a structural model for long-term potentiation. *Physiol. Rev.* **75**, 759–787 (1995).
- Bekkers, J. M. & Stevens, C. F. NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus. *Nature* **341**, 230–233 (1989).
- Liu, G. & Tsien, R. W. Properties of synaptic transmission at single hippocampal synaptic boutons. *Nature* **375**, 404–408 (1995).
- Lewis, C. A. & Faber, D. S. Inhibitory synaptic transmission in isolated patches of membrane from cultured rat spinal cord and medullary neurons. *J. Neurophysiol.* **76**, 461–470 (1996).
- Fatt, P. & Katz, B. Spontaneous subthreshold activity at motor nerve endings. *J. Physiol. (Lond.)* **117**, 109–128 (1952).
- Betz, W. J. & Bewick, G. S. Optical analysis of synaptic vesicle recycling at the frog neuromuscular junction. *Science* **255**, 200–203 (1992).
- Ryan, T. A. et al. The kinetics of synaptic vesicle recycling measured at single presynaptic boutons. *Neuron* **11**, 713–724 (1993).
- Stuehmer, W., Roberts, W. M. & Almers, W. in *Single Channel Recording* (eds Sakmann, B. & Neher, E.) 123–132 (Plenum, New York, 1983).
- Spruston, N., Jonas, P. & Sakmann, B. Dendritic glutamate receptors channels in rat hippocampal CA3 and CA1 pyramidal neurons. *J. Physiol. (Lond.)* **482**, 325–352 (1995).
- Jonas, P., Major, G. & Sakmann, B. Quantal components of unitary EPSCs at the mossy fiber synapse of CA3 pyramidal cells of rat hippocampus. *J. Physiol. (Lond.)* **472**, 615–663 (1993).
- Edwards, F. A., Konnerth, A. & Sakmann, B. Quantal analysis of inhibitory synaptic transmission in the dentate gyrus of rat hippocampal slices: a patch-clamp study. *J. Physiol. (Lond.)* **430**, 213–249 (1990).
- Silver, R. A., Traynelis, S. F. & Cull-Candy, S. G. Rapid-time-course miniature and evoked excitatory currents at cerebellar synapses *in situ*. *Nature* **355**, 163–166 (1992).
- Malgarioli, A. & Tsien, R. W. Glutamate-induced long term potentiation of the frequency of miniature synaptic currents in cultured hippocampal neurons. *Nature* **357**, 134–139 (1992).
- Larkman, A., Stratford, K. & Jack, J. Quantal analysis of excitatory synaptic action and depression in hippocampal slices. *Nature* **350**, 344–347 (1991).
- Stricker, A. C., Field, A. C. & Redman, S. J. Statistical analysis of amplitude fluctuations in EPSCs evoked in rat A1 pyramidal neurones *in vitro*. *J. Physiol. (Lond.)* **490**, 419–441 (1996).
- Kullmann, D. M. & Nicoll, R. A. Long-term potentiation is associated with increases in quantal content and quantal amplitude. *Nature* **357**, 240–244 (1992).
- Liao, D., Jones, A. & Malinow, R. Direct measurement of quantal changes underlying long-term potentiation in A1 hippocampus. *Neuron* **9**, 1089–1097 (1992).
- Bekkers, J. M., Richerson, G. B. & Stevens, C. F. Origin of variability in quantal size in cultured hippocampal neurons and hippocampal slices. *Proc. Natl Acad. Sci. USA* **87**, 5359–5362 (1990).

- Korn, H., Sur, C., Charpier, S., Legendre, P. & Faber, D. S. in *Molecular and Cellular Mechanisms of Neurotransmitter Release* (eds Stjärne, L. et al.) 301–322 (Raven, New York, 1994).
- Bauerfeind, R., Hüttner, W. B., Almers, W. & Augustine, G. J. Quantal neurotransmitter release from early endosomes? *Trends Cell Biol.* **4**, 155–156 (1994).
- Jack, J. J. B., Noble, D. & Tsien, R. W. *Electric Current Flow in Excitable Cells* (Oxford Univ. Press, Oxford, UK, 1983).
- Spruston, N., Jaffe, D. B., Williams, S. H. & Johnston, D. Voltage- and space-clamp errors associated with measurement of electrotonically remote synaptic events. *J. Neurophysiol.* **70**, 781–802 (1993).
- Faber, D. S., Young, W. S., Legendre, P. & Korn, H. Intrinsic quantal variability due to stochastic properties of receptor–transmitter interactions. *Science* **258**, 1494–1498 (1992).
- Hestrin, S. Activation and desensitization of glutamate-activated channels mediating fast excitatory synaptic currents in the visual cortex. *Neuron* **9**, 991–999 (1992).
- Silver, R. A., Cull-Candy, S. G. & Takahashi, T. Non-NMDA glutamate receptor occupancy and open probability at a rat cerebellar synapse with single and multiple release sites. *J. Physiol.* **494**, 231–250 (1996).
- Ferking, M. & Wilson, M. Saturation of postsynaptic receptors at central synapses? *Curr. Opin. Neurobiol.* **6**, 395–403 (1996).
- Liao, D., Hessler, N. A. & Malinow, R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375**, 400–404 (1995).
- Isaac, J. T. R., Nicoll, R. A. & Malenka, R. C. Evidence for silent synapses: implications for the expression of LTP. *Neuron* **15**, 427–434 (1995).
- Durand, G. M., Kovalchuk, Y. & Konnerth, A. Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* **381**, 71–75 (1996).

Acknowledgements. We thank D. DiFrancesco, D. S. Faber, A. Marty, J. Meldolesi and R. W. Tsien for comments on the manuscript, and A. Abenavoli, L. Anghinoni, M. Caimi, P. Cerutti, E. Naldi and J. Noel for help and discussion. L.F. was the recipient of a postdoctoral fellowship from the University of Milano. This research was supported by grants from EC Biomed-2, Telethon and PNR/Neurobiology (MURST-Italy) to A.M.

Correspondence and requests for materials should be addressed to A.M. (e-mail: malgara@dibit.hsr.it).

Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E

Steven W. Barger*†‡ & Ashley D. Harmon†

* Donald W. Reynolds Department of Geriatrics, and † Department of Anatomy, Cell Biology, and Neurobiology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, USA

Geriatric Research Education and Clinical Center, John L. McClellan Memorial Veterans Affairs Medical Center, Little Rock, Arkansas 72205, USA

A role for β -amyloid precursor protein (β -APP) in the development of Alzheimer's disease has been indicated by genetics¹, and many conditions in which β -APP is raised have been associated with an increased risk of Alzheimer's disease or an Alzheimer's-like pathology^{2–4}. Inflammatory events may also contribute to Alzheimer's disease⁵. Here we investigate whether a secreted derivative of β -APP (sAPP- α) can induce inflammatory reactions in microglia, which are brain cells of monocytic lineage. We found that treatment with sAPP- α increased markers of activation in microglia and enhanced their production of neurotoxins. The ability of sAPP- α to activate microglia was blocked by prior incubation of the protein with apolipoprotein E3 but not apolipoprotein E4, a variant associated with an increased risk for Alzheimer's⁶. A product of amyloidogenic β -APP processing (sAPP- β) also activated microglia. Because sAPP- β is deficient in the neuroprotective activity shown by sAPP- α , our results indicate that increased amyloidogenic processing could adversely affect the balance of sAPP activities that determine neuronal viability.

To determine the impact of sAPP on inflammatory reactions in microglia, we treated the N9 microglial cell line⁷ with sAPP and measured the activity of the transcription factor NF- κ B by electrophoretic mobility-shift assay (EMSA) (Fig. 1). These cells responded to sAPP with activation of NF- κ B within 90 min. Induction of a κ B-binding transcription factor by sAPP in primary neurons is dependent upon an increase in the concentration of cyclic GMP⁸ and involves a transcription factor distinct from NF- κ B (unpublished