Fast presynaptic $GABA_A$ receptor-mediated Cl^- conductance in cultured rat hippocampal neurones

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1. Hippocampal neurones cultured from the 18-day-old embryonic rat for 3 days to 3 weeks were recorded with Cl^--filled patch pipettes. Spontaneous synaptic currents, which reversed at the equilibrium potential for Cl^- ions (E_{Cl}) and were blocked by the $GABA_A$ (γ-aminobutyric acid) receptor antagonists bicuculline or picrotoxin, were recorded in every culture. At 25 °C and −80 mV they decayed with a time constant $\geqslant$ 20 ms that invariably increased at positive potentials. After 2 weeks, 50–75% of all neurones were GABA immunoreactive.

2. In pairs-recordings, coincident synaptic currents in both cells were either spontaneous or evoked by stimulation of one cell. In the presence of tetrodotoxin and using pipettes containing lidocaine (lignocaine) *N*-ethyl bromide, coincident spontaneous Cl^- transients still occurred in both neurones far more frequently than expected by chance.

3. Holding the potential of one neurone at a positive value reversed the synaptic transients in that cell and, in half of the cells, increased the frequency of coincident events in both cells.

4. In neurones where depolarization increased the frequency of coinciding events and all regenerative current apparent at the soma was abolished, short depolarizing pulses occasionally evoked all-or-none, pre- and postsynaptic currents with matching transmission failures and identical delays in transmission.

5. The results suggest that the same pulse of GABA simultaneously activates $GABA_A$ receptor-coupled Cl^- channels on both sides of the same synaptic cleft, producing immediate auto-transmission in the absence of collaterals or interneurones.

It is known that chemically mediated fast synaptic transmission is polarized: transmitter is released transiently from the terminal of the presynaptic neurone and activates specific ionic conductances in the membrane of the postsynaptic neurone (Fig. 1*A*). This contrasts with electrical synapses which, with a few exceptions, are not polarized and can conduct transient currents in either direction. The physiological polarization of chemical synapses is consistent with the asymmetry of their morphological organization (Couteaux, 1958; Birks, Huxley & Katz, 1960; Heuser & Reese, 1977). Typically, a group of synaptic vesicles is present at presynaptic sites, while receptors specific to the presynaptic neurotransmitter are detected at the postsynaptic membrane. Presynaptically located receptors have also been identified throughout the nervous system, including the hippocampus, where a second messenger seems to be involved in coupling these presynaptic receptors to their effectors (Thomson, Capogna & Scanziani, 1993).

Rapid presynaptic conductances have also been reported at chemical synapses with a symmetrical structure (Horridge, Chapman & Mackay, 1962) (Fig. 1*D*), which have been found in invertebrates (Anderson & Grünert, 1988) as well as in vertebrates (Kohno, 1970). These synapses differ from reciprocal synapses (Prince & Powell, 1970) (Fig. 1*B*), where the vesicle clumps are offset from one another and represent side-by-side synapses transmitting in opposite directions. At truly symmetrical synapses supporting bidirectional transmission, each presynaptic terminal also exhibits a postsynaptic function (Fig. 1*D*). Thus, the transmitter originating from one neurone generates simultaneous synaptic signals in both pre- and postsynaptic neurones. In addition to the typical pre- to postsynaptic hetero-transmission, the existence of symmetrical synapses implies that a form of purely presynaptic auto-transmission may also function (Fig. 1*E*). Such same-sided transmission contrasts with 'autaptic' auto-transmission, which refers to a synaptic

contact between a neurone's collateral and one of its own neurites or its soma (Van der Loos & Glaser, 1972; Bekkers & Stevens, 1991; Segal, 1991) where presynaptic terminal and postsynaptic membranes remain specialized, separated by the synaptic cleft and functionally polarized (Fig. 1*C*).

Bekkers & Stevens (1991) and Segal (1991) recorded synaptic γ-aminobutyric acid$_A$ ($GABA_A$) receptor-mediated auto-transmission in cultures containing one hippocampal neurone, which they attributed to autaptic contacts. However, they could not distinguish between autaptic GABAergic transmission from a collateral across a synaptic cleft and GABA acting back on the terminal neuritic membrane. Furthermore, Benveniste, Harrison, Bekelmam & Barker (1989) have shown that cultured neuronal networks composed of hippocampal cells exhibit fast $GABA_A$-mediated presynaptic inhibition that involves recurrent auto-transmission. All of these results suggest the presence of autaptic contacts in cultured hippocampal neurones. However, since it has been demonstrated that $GABA_A$ receptors are present on presynaptic terminals in different parts of the brain (Floran, Silva, Nava & Aceves, 1988; Zhang & Jackson, 1993) including the hippocampus (Richards, Schoch, Häring, Takacs & Möhler, 1987), another form of fast auto-transmission might directly and immediately engage $GABA_A$ autoreceptor-coupled Cl^- channels without an intervening collateral (Fig. 1*E*). We investigated auto-transmission in synaptically coupled pairs of cultured rat hippocampal neurones and we report that GABA mediates spontaneously occurring, precisely coincident miniature synaptic signals. The coincidence of pre- and postsynaptic miniature signals denotes the presence of autoreceptors. We call this form of same-sided auto-transmission 'cis-mission' to distinguish it from autaptic auto-transmission involving collaterals.

METHODS

Pregnant Sprague–Dawley (Taconic farms, Germantown, NY, USA) female rats were killed by CO_2 inhalation. Eighteen-day-old embryos were removed and immediately decapitated. Hippocampi were enzymatically dissociated with papain (Worthington, Freehold, NJ, USA). Cells were cultured in 35 mm plates coated with 20 μg ml^{-1} high molecular weight poly-D-lysine at a density of $2{\cdot}1\times10^4$ to $5{\cdot}2\times10^4$ cells cm^{-2}. Plating medium consisted of minimal essential medium (MEM) with 3·7 g l^{-1} sodium bicarbonate, 6 g l^{-1} glucose (Gibco), 5% fetal calf serum and 5% horse serum. Cultures were kept at 36 °C in a CO_2 incubator and after 4 days were maintained with MEM and 5% horse serum. The presence of GABA was determined after fixation by

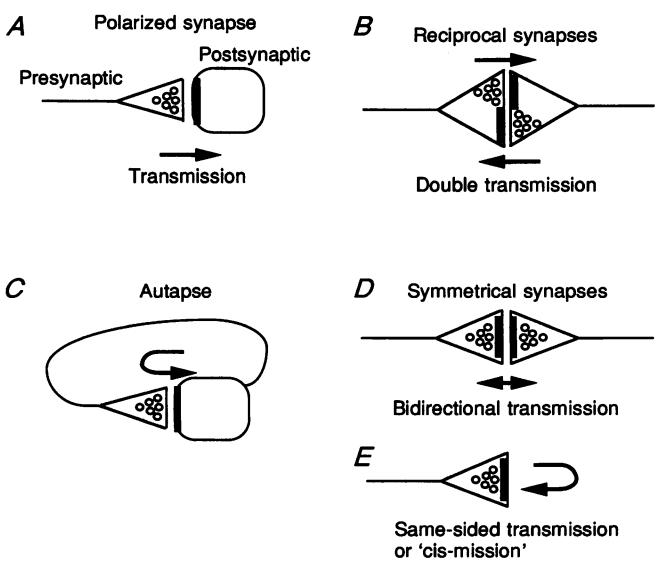

Figure 1. Classification of chemically mediated synaptic transmission
A, diagrammatic representation of common models of synaptic transmission. Transmission polarization is due to the specialization of pre- and postsynaptic elements. Vesicles are clustered near the release site of the presynaptic terminal. The postsynaptic membrane shows electron-opaque material where specific receptors are located. *B*, reciprocal synapses convey transmission in both directions at two closely juxtaposed polarized synapses. *C*, at autapses, a collateral contacts the cell from which it issues and transmission is still polarized according to pre- and postsynaptic specializations which, because they are on the same cell, convey auto-transmission. *D*, there is morphological and physiological evidence for symmetrical synapses (see text). Pre- and postsynaptic specializations are present at the same side of the synaptic cleft. *E*, auto-transmission at symmetrical synapses contrasts with polarized, autaptic auto-transmission in that it does not involve a collateral and is not polarized across a synaptic cleft. Here, we have termed such 'same-sided' auto-transmission as 'cis-mission'.

indirect immunocytofluorescence using methods established previously (Schaffner, Behar, Nadi & Barker, 1993). Postsynaptic currents were recorded at room temperature (22–25 °C) using two independent patch clamp amplifiers (List Electronic L/M-EPC7, Damstadt-Eberstadt, Germany). The patch pipettes for electrical recording contained (mM): CsCl, 145; $CaCl_2$, 0·1; EGTA, 1·1; $MgCl_2$, 2; ATP, 5; and Hepes, 5, adjusted to pH 7·2. Osmolarity was adjusted to 310 mosmol l^{-1} with sucrose. Occasionally Lucifer Yellow dye (Sigma, St Louis, MO, USA) or 1–10 mM lidocaine *N*-ethyl bromide (QX-314; Research Biomedicals Inc., Natick, MA, USA) were added to the pipette saline. The bath contained (mM): NaCl, 115; $NaHCO_3$, 25; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; D-glucose, 10; and Hepes, 10, adjusted to pH 7·4. Osmolarity was adjusted to 295 mosmol l^{-1}. Tetrodotoxin (TTX; Sigma T8024; 1–5 μM) was eventually added to the chamber. About one-third of the eighty neurones included in the study exhibited spontaneous synaptic currents, all of which reversed polarity at approximately −7 mV (the equilibrium potential for Cl^-, E_{Cl}) and, at −80 mV, had a decay that could be fitted satisfactorily by an exponential with a time constant $\geqslant$ 20 ms, which became longer at more positive holding potentials (see Fig. 5*Ba* and *Bb*). Either of the classical GABA receptor antagonists bicuculline (100 μM) or picrotoxin (10 μM) blocked all of this spontaneous synaptic activity, which we therefore conclude is mediated by GABA (Barker & Harrison, 1988; Vautrin, Schaffner & Barker, 1992). Clamping currents from both neurones were digitally recorded at 9 kHz on a four-channel VCR PCM system (Instrutech VR-100A, Elmont, NY, USA). Transient Cl^- currents from both neurones were low-pass filtered at 1–3 kHz (8-pole Bessel) and monitored simultaneously on a memory oscilloscope (Tektronix 7313) with both 0·1 Hz and 0·1 kHz high-pass filtering. High-pass filtering of the analog signals at 0·1 kHz differentiated them, producing a spike in the discrete differentiation of the current over time ($\Delta I/\Delta t$) at the precise moment the slope of the rising phase in the raw signal reached its maximum (analog signal not shown). Synaptic signals from both neurones were simultaneously digitized (12 bits, National Instrument LAB-PC card) at 5–10 kHz using a DC or 0·1 kHz high-pass

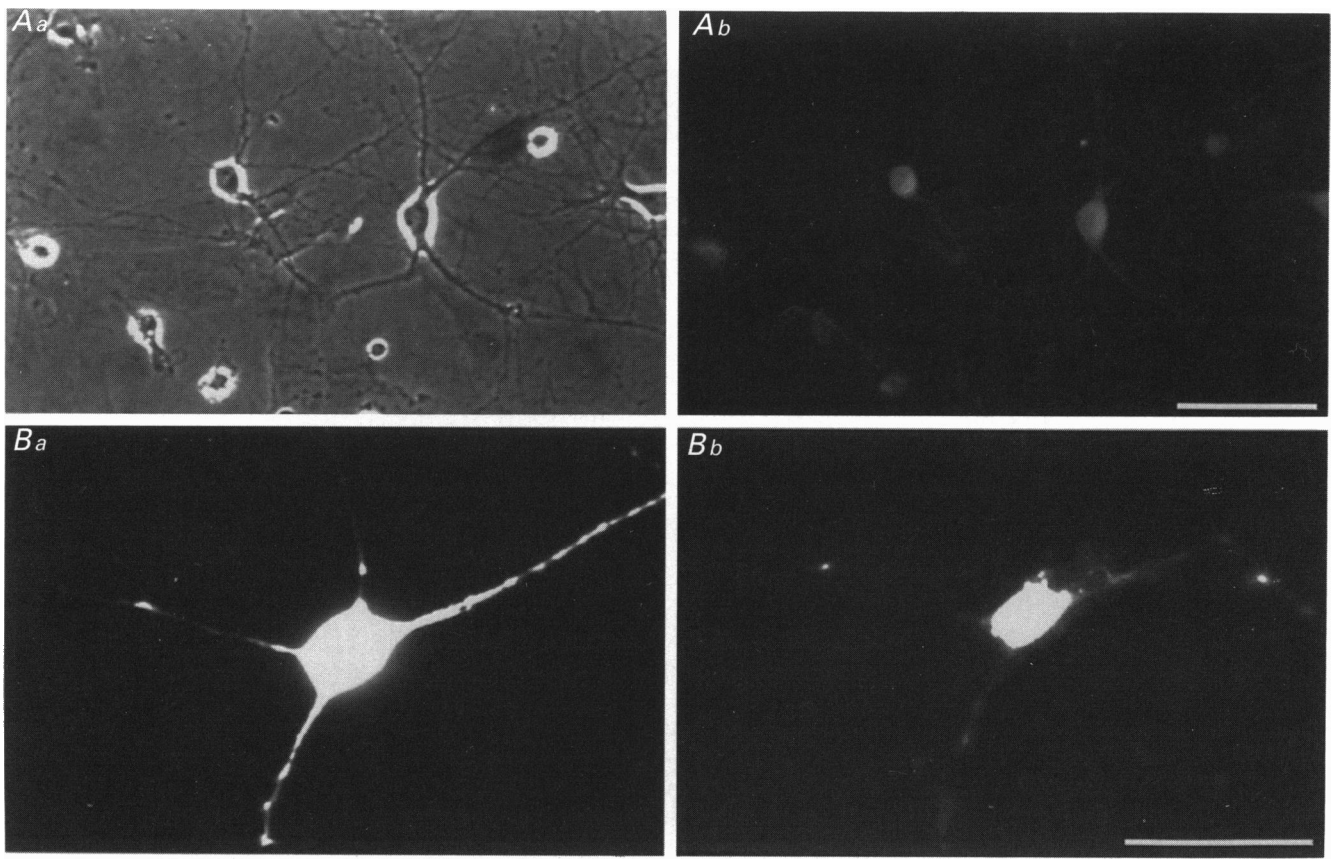

Figure 2. Cultured rat hippocampal neurones are GABA immunoreactive
Phase contrast (*A a*) and GABA-IR (GABA immunoreactivity) (*A b*) of the same field of hippocampal neurones after 2 weeks in culture. Most of the cells exhibit GABA-IR. Scale bar represents 100 μm. *Ba*, fluorescence micrograph of a neurone after being loaded with Lucifer Yellow during recording by inclusion of the dye in the patch pipette. *Bb*, fluorescence microscopy of the neurone shown in *Ba* after fixation, photographed at another wavelength showing GABA-IR. Scale bar represents 50 μm.

connection and analysed using SPAN, SCAN and VCAN programs (courtesy of J. Dempster, Strathclyde University, Glasgow, UK). Synaptic transient signals were eventually numerically differentiated over 1 ms ($\Delta I/\Delta t = I_t - I_{t+1\,ms}$) to identify the instant of maximum rate of rise in both neurones.

RESULTS

General observations

More than 50% of the cells at 1 week in culture and more than 75% at 2 weeks (Fig. 2*A a* and 2*A b*) exhibited GABA immunoreactivity (IR) that was diffusely distributed throughout cell bodies and processes (Fig. 2*B b*). Fluorescence microscopic inspection of cells recorded with pipettes containing Lucifer Yellow ($n > 30$) revealed that the dye always remained restricted to the patched neurone without ever diffusing either to a recorded or unrecorded neighbouring cell (Fig. 2*B a*). The high percentage of GABA-IR neurones in culture leads to a high probability of recording from GABA-IR neurones (Fig. 2*B b*).

$GABA_A$ receptor-activated synaptic transient Cl^- currents were routinely recorded in 3 day to 3 week cultures (Vautrin & Kriebel, 1991; Vautrin, Schaffner & Barker, 1993). Two pipettes and two patch clamp systems were used to record simultaneously the synaptic activities at −80 mV in two neurones whose arborizations overlapped. Equilibrating the cell interior by whole-cell recording with patch pipettes containing a high Cl^- concentration led to a depolarized reversal potential for bicuculline-sensitive

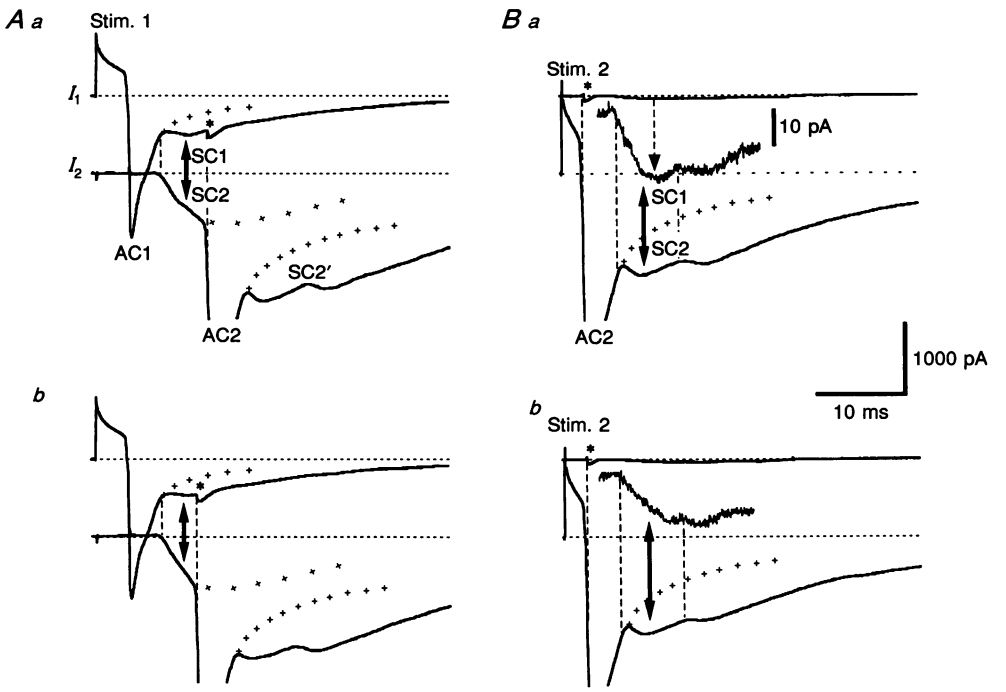

Figure 3. Synaptic currents simultaneously evoked by current pulses in pairs of neighbouring cultured hippocampal neurones

The neurones are clamped at −80 mV and stimulated alternately with short pulses (up to +100 mV depolarization) in the absence of voltage-gated channel blockers. Horizontal dashed lines represent baseline levels of holding current in each neurone. Crosses are suggestions of the boundaries between the different components of the signals. Vertical dashed lines show coinciding events. The artifacts caused by the action currents in the communicating cell are marked by an asterisk (*). *A a* and *A b* are two sets of pairs-recording traces showing a stimulation of neurone 1 (Stim. 1) immediately followed by an action current in neurone 1. Each action current in neurone 1 evokes a synaptic current in neurone 2 (SC2) as well as a synaptic current in neurone 1 (SC1) at the same delay, which suggests the presence of auto-transmission. The synaptic current evoked in neurone 2 (SC2) triggers an action current in neurone 2 (AC2, truncated), which in turn evokes a second synaptic current in that neurone (SC2′). *B a* and *B b*, two sets of pairs-recording traces showing a stimulation (Stim. 2) of neurone 2 of the same pairs as in *A a* and *A b*, immediately followed by an action current in neurone 2 (AC2), which evokes in turn a synaptic current in that neurone (SC2). Higher amplification of the holding current in neurone 1 (20 pA calibration rather than 1000 pA) reveals a small synaptic current in neurone 1 (SC1) that coincides with the auto-transmitted synaptic current in neurone 2 (SC2).

synaptic currents. In the absence of voltage-gated channel blockers, network-driven or stimulus-evoked GABA-mediated synaptic currents were large and fast enough (500–1500 pA; see also Bekkers & Stevens, 1991) to trigger inward regenerative currents resembling action potentials (Fig. 3*Aa* and *Ab*). Synaptic transients were not only evoked in the postsynaptic element but also often in the presynaptic neurone (Fig. 3). Occasionally, these

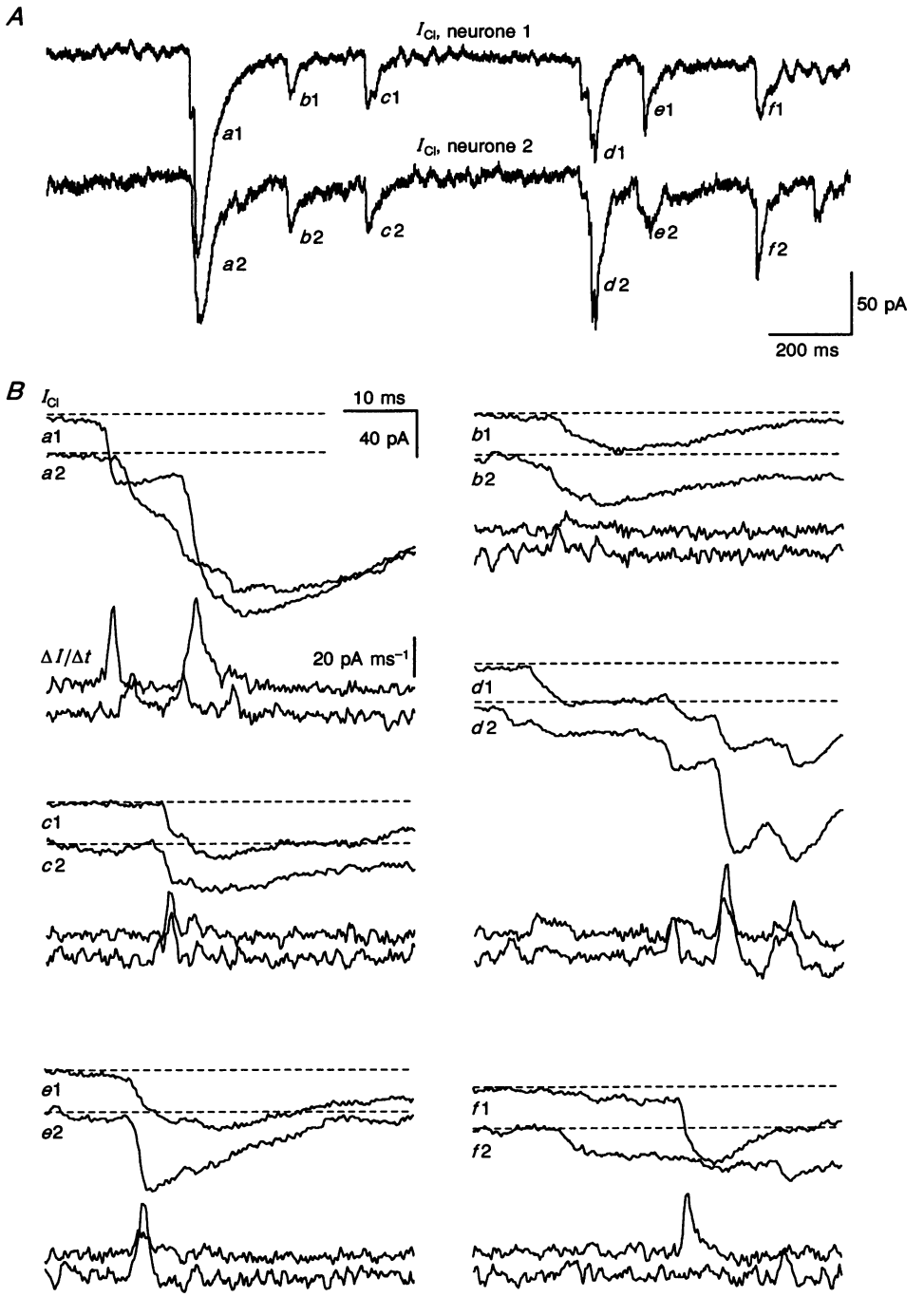

Figure 4. Coincident miniature synaptic Cl^- conductances in pairs of cultured hippocampal neurones

A, transient Cl^- currents often coincided in spite of the presence of 1 μM TTX in the external medium and 10 mM QX-314 in the pipette saline. Coinciding events are denoted *a*1 and *a*2, *b*1 and *b*2, etc. *B*, the upper pair of each set of traces details the time courses of transient Cl^- currents displayed in *A*. The lower pair describes the corresponding numerical differentiation of the current signal.

presynaptic currents triggered action potential-like currents. If this occurred in an axon terminal rather than at dendro-dendritic synapses, which could not be distinguished in living neurones examined using phase-contrast microscopy, then the backfiring should be characterized as antidromic. In turn, this backfiring activity evoked transmitter release leading to paroxysmal bursts of synaptic and regenerative transients in both cells, which, after three to ten action currents, stopped spontaneously before a new burst started a few seconds later (not shown).

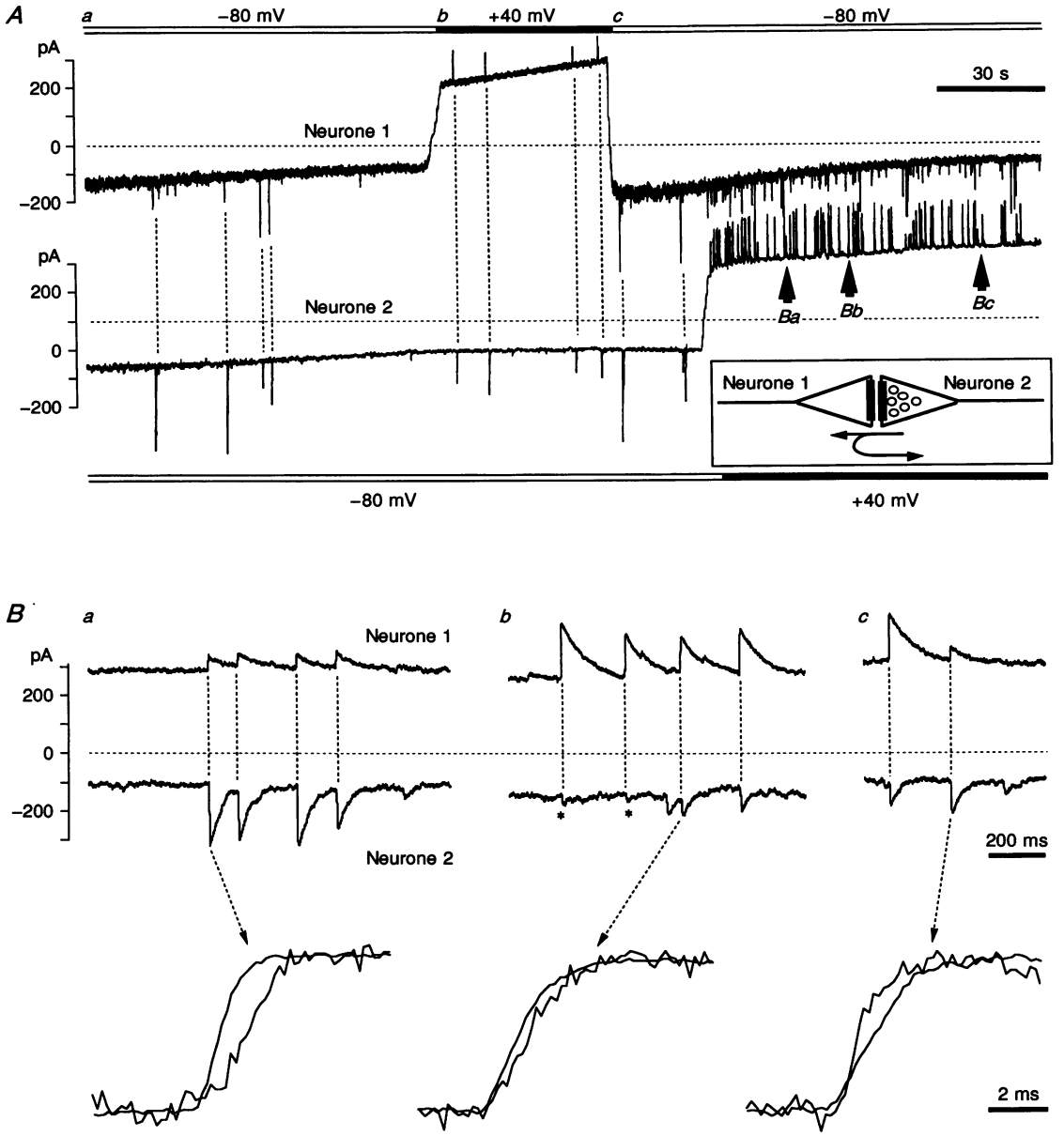

Figure 5. Depolarization-induced increase in the frequency of coincident Cl^- transients
A, typical simultaneous recording of clamping currents in two neighbouring neurones. *Aa*, both neurones have initially been clamped at −80 mV. Dashed lines indicate coincident transient Cl^- currents. *Ab*, holding potential of neurone 1 is moved to +40 mV and the transient currents reverse polarity but do not change in frequency. *Ac*, holding potential of neurone 2 is moved to +40 mV and transient Cl^- currents reverse polarity. The frequency of the transient currents, most of which are coincident, increases in both neurones. Inset is a diagrammatic representation of the functional synaptic connections between the two neurones illustrated in this figure. *B*, details of episodes marked *Ba*, *Bb* and *Bc* in *A*. Dots in *Bb* point to transient Cl^- currents in neurone 1 that would have gone unnoticed if there were not matching transient currents in neurone 1. For each episode, the rising phases of a coincident pair of transient currents has been resolved on a faster time scale and amplitudes adjusted in order to compare the time courses.

Coincident, spontaneous synaptic transients

In the presence of 1 μM TTX in the medium and 1–10 mM QX-314 in both patch pipettes, it was impossible to evoke regenerative activity in either of the connected neurones using depolarizing commands. Furthermore, all the large-amplitude (500–1500 pA) synaptic currents were blocked. In spite of these pharmacological effects to uncouple neurones, up to 90% of the spontaneous synaptic transients occurred synchronously in both neurones (Fig. 4*A*). Rising phases coincided precisely or within a few milliseconds. Falling phases could be either apparently similar or significantly different (not shown). Presumably, in the presence of TTX and QX-314, two potent voltage-dependent Na^+ channel blockers that eliminate Na^+-dependent regenerative activity propagated in neuronal networks, synaptic transmission is restricted to the spontaneous release of asynchronous quanta. However, transient Cl^- currents in the two cells manifestly coincided more often than expected by chance. The ratio between the amplitudes of the signals recorded in the pair could vary by up to a factor of ten. Individual amplitudes and rise times (measured between 10 and 90% of peak amplitude) tended to correlate in both neurones (Fig. 4*A*). Cl^- transients with relatively shorter rise times (1–2 ms) and 20–40 pA peak amplitudes (250–500 pS) showed onsets and slope maxima that coincided precisely (Fig. 4*Bc*1, *Bc*2, *Ce*1 and *Ce*2). Transient Cl^- currents with relatively longer rise times showed complex rising phases and the corresponding differentiated signals exhibited multiple spikes that remained synchronous in many cases (Fig. 4*Bd*1 and *Bd*2).

Depolarization-induced coincident transients

When the holding potential in one neurone of the pair recorded in TTX and QX-314 was moved to a positive value, typically +40 mV, Cl^- transients reversed polarity (Fig. 5*A*). Coincidences between events in the two neurones persisted and the positive holding potential of one or both neurones increased the frequency of the coinciding transients, thus revealing which neurone(s) of the recorded pair was (were) functionally presynaptic. Pairs were recorded with comparable frequency in which either one or both of the elements was transmitting and detecting GABA signals.

The ratio of the amplitudes recorded in the two cells varied from pair to pair and from one flurry of spontaneous Cl^- transients to another (Fig. 5*Ba* and *Bb*) or from one Cl^- transient to another (Fig. 5*Bc*).

Two classes of spontaneous $GABA_A$-mediated miniature synaptic currents have previously been recorded in these neurones (Vautrin *et al.* 1992). The small mode is generally predominant at −80 mV in the presence of 1 μM TTX, while the transient current contributing to the increase in frequency associated with depolarization belonged mainly to the large-mode class. Onset and rising phases of the transients were generally superimposable and differed by less than 1 ms.

Stimulus-evoked coincident transients in TTX

TTX and QX-314 eliminated or decreased drastically the 500–1500 pA stimulus-evoked Cl^- transients, yet even after all voltage-dependent regenerative currents detected in the soma were abolished (see Figs 5 and 6), it was sometimes still possible to evoke all-or-none, quantal-like transient Cl^- currents using 100–500 pA pulses (up to +100 mV voltage commands) of 3–20 ms duration (Fig. 6). Such stimulus-evoked transient Cl^- currents belonged mainly to the large-mode class (Vautrin *et al.* 1992). The proportion of transmission failures generally increased over time, leading eventually to complete transmission block, but it was usually possible to recover evoked transmission by progressively increasing the stimulus intensity. Evoked transient Cl^- currents exhibited rather constant amplitudes (50–200 pA) but very variable delays that could be as long as 100 ms and depended on the stimulus intensity and duration. More importantly, when transient Cl^- currents were evoked in both recorded neurones, synaptic delays as well as transmission failures were matching in the two recorded neurones (Fig. 6). Furthermore, rising phase durations (illustrated by the spike width in $\Delta I/\Delta t$ signals) coincided precisely (Fig. 6*Bc* and *Bd*) or within 2 ms (Fig. 6*Ba*).

DISCUSSION

Coincident synaptic transients are not due to electrical coupling

Coincident Cl^- transients recorded in pairs of neurones could be due to electrical coupling; however, the dye Lucifer Yellow never diffused from an injected neurone to any neighbouring one. Furthermore, the time course, and particularly the falling phases of coincident TTX- and QX-314-resistant synaptic current, were sometimes different in the two neurones of any recorded pair. More importantly, no evidence of electrical coupling between the neurones of the pairs was observed when injecting short or long hyperpolarizing or depolarizing currents. In fact, Cl^- transients could be reversed in a neurone without affecting those in the other neurone recorded, which demonstrates that the polarity of the transient currents depended on the Cl^- driving force existing at the membrane of the very neurone where they were generated. Thus, we conclude that they were chemically mediated rather than electrically transmitted.

Asynchronous 'miniature' transients coincide in synaptically connected pairs

TTX and QX-314 are each considered sufficient to block voltage-sensitive Na^+ channels and Na^+ action potentials supporting the excitation of the whole neurone. Since they

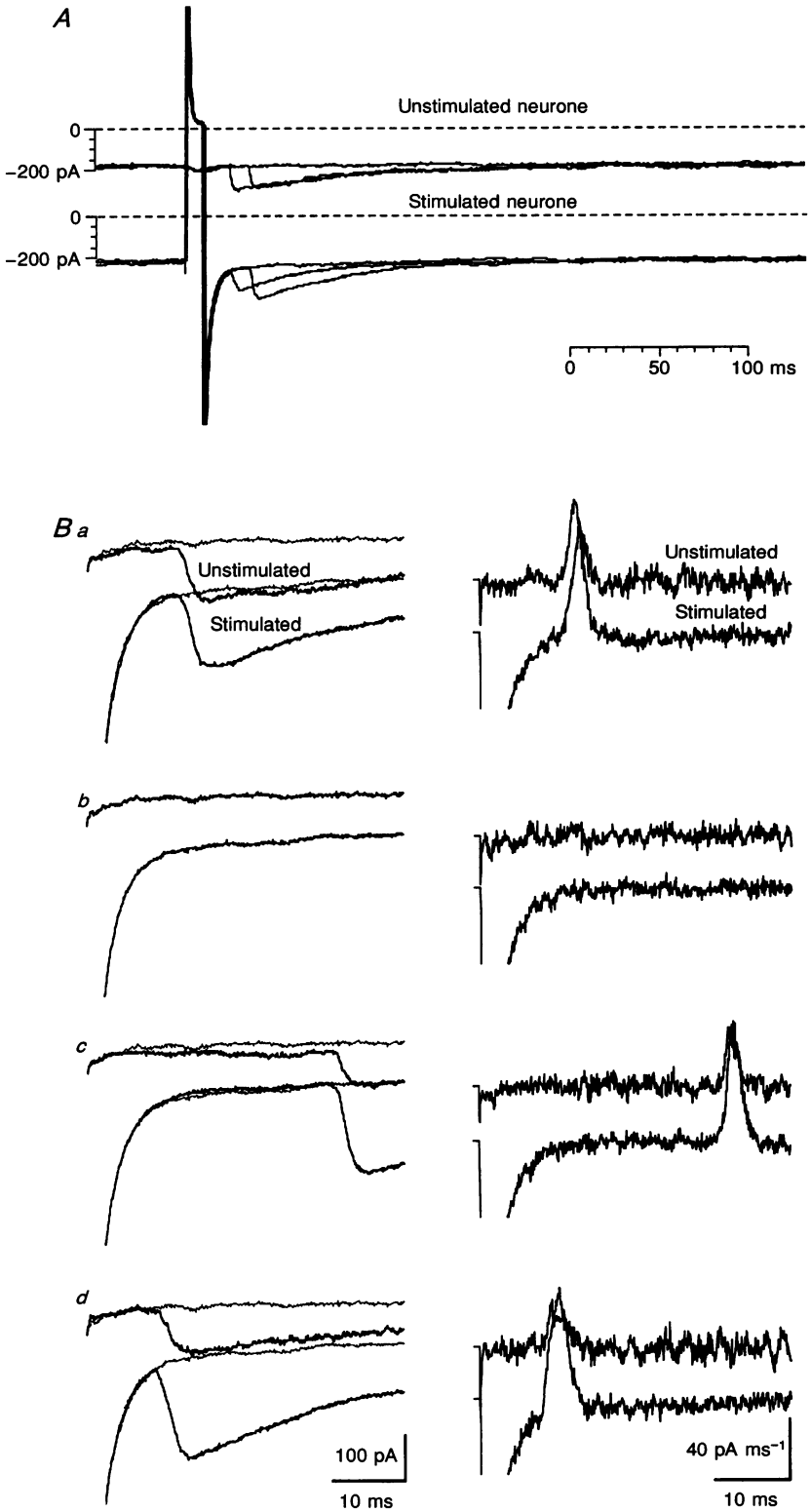

Figure 6. TTX- and QX-314-resistant, stimulation-induced quantal synaptic Cl^- currents
A, three superimposed current traces in two neighbouring neurones clamped at −80 mV, one being stimulated with 10 ms pulses to +80 mV. There is no regenerative inward current during the 10 ms pulse, but coincident transients are triggered after two of the three stimuli. *Ba–Bd*, details of the current traces (left panels) and their differentiation (right panels) corresponding to four successive stimuli. *Bb* is a failure of transmission, which is also superimposed on the other traces *Ba*, *Bc* and *Bd*. When triggered, the rising phase of the transients in *Bc* and *Bd* are precisely coincident.

abolish propagated depolarizations spontaneously occurring in neuronal networks, these drugs are believed to prevent synchronized transmitter release from presynaptic sites scattered in the neuritic arborization. This is why TTX-resistant synaptic transients have been identified as 'miniature' (Ropert, Miles & Korn, 1990; Edwards, Konnerth & Sakmann, 1990; but see also Llano & Gerschenfeld, 1993) and compared with miniature endplate potentials recorded at neuromuscular preparations in the absence of stimulation of the presynaptic axon (Fatt & Katz, 1952). Miniature synaptic signals are commonly ascribed to elementary transmission caused by the spontaneous and random release of transmitter quanta. Since they are released randomly and spontaneously from one of the recorded neurones or from other neurones in the network, they are expected to be asynchronous and, consequently, highly unlikely to coincide when occurring at moderate frequencies, as recorded here.

The presence of QX-314 in both patch pipettes makes it even more unlikely that the coincident Cl^- currents could result from the simultaneous release at two collateral terminals of the stimulated neurone, one projecting onto itself, the other onto the second neurone recorded; however, the depolarization of one or both of the recorded neurones increased the frequency of coinciding synaptic events. The quantal-like synaptic transients evoked in both neurones when stimulating one of them in the presence of extracellular TTX and intracellular QX-314 appears rather surprising. It would be difficult, if not impossible, to conclude that all voltage-gated Na^+ or Ca^{2+} conductances in the plasma membrane have been blocked in these conditions. However, the duration (up to tens of milliseconds) and variability in their latencies suggest that these transients may not involve the same release process as action potential-evoked transmitter release. Rather, this mode of TTX- and QX-314-resistant transmission resembles the increased frequency of asynchronous release induced by long-lasting depolarizations, and may represent a more diffuse increase of Ca^{2+} concentration than the voltage-induced focal Ca^{2+} entry. Although the mechanism by which brief depolarizations occasionally induce transient release in TTX and QX-314 is uncertain, it is striking that despite the large variability in their latency from one stimulus to the next, the latencies are virtually identical in both neurones recorded.

Coincident synaptic transients reflect transmission and cis-mission

These results suggest that coincident TTX- and QX-314-resistant Cl^- transients that are recorded simultaneously in two electrically isolated neurones are the consequence of the same elementary transmitter release. Such transients could only coincide if both of the receptor-bearing membranes are at the release site. Since these elementary releases can be evoked by depolarization or brief pulses in one of the neurones where they are recorded, this neurone must fulfil both presynaptic and postsynaptic functions. Such auto-transmission, often recorded in cultured hippocampal neurones (Stasheff, Mott & Wilson, 1993), would not be conveyed by an autaptic circuit as proposed by Benveniste *et al.* (1989), Bekkers & Stevens (1991) and Segal (1991), since in this case there is evidence that another neurone already fills the postsynaptic position. Rather, coincident TTX- and QX-314-resistant synaptic transients recorded in two different neurones, one of which at least functions as the presynaptic element, constitute evidence for a 'same-sided' form of auto-transmission or cis-mission. Thus, it is not clear if the auto-inhibition recorded in single-neurone cultures (Bekkers & Stevens, 1991) is due to autaptic transmission or cis-mission. Non-coincident synaptic transients in the same recordings (Fig. 3*A*) may simply represent spontaneous release from terminals of (an)other neurone(s) in the culture or from the terminal(s) of one neurone of the pair recorded that do(es) not bear $GABA_A$ receptors and supports only simple transmission.

This demonstration of cis-mission relies on the assumption that a miniature synaptic transient recorded in the absence of presynaptic action current follows from the almost instantaneous and random release by exocytosis of the transmitter stored in a presynaptic vesicle (Fatt & Katz, 1952; Del Castillo & Katz, 1955). According to this representation, miniature synaptic signals are all or none and represent the most elementary form of transient transmitter release. However, this model may be oversimplified since evidence suggests that TTX- and QX-314-resistant GABAergic synaptic currents recorded in networks of hippocampal neurones, like miniature endplate currents at the neuromuscular junction (Erxleben & Kriebel, 1988), have a substructure and are actually composed of subunits. Ropert *et al.* (1990) have shown that there are more short intervals between GABAergic miniature Cl^- currents in hippocampal slices than expected from random occurrence, suggesting short-term interactions between spontaneous transmitter releases. Interdependent transmitter releases occurring at intervals shorter than the rise time of the elementary postsynaptic response integrate postsynaptically and produce complex synaptic signals with variable amplitudes and rise times (see Vautrin & Kriebel, 1991; Vautrin *et al.* 1993). Indeed, Edwards *et al.* (1990) have noticed that frequency distributions of hippocampal GABAergic miniature signal amplitudes may show peaks, indicating that they are caused by a variable number of elementary transmitter releases. This is consistent with the multiple spikes on the differentiated rising phase of the larger and generally slower TTX- and QX-314-resistant synaptic Cl^- currents (Fig. 4*Ba*1, *Ba*2, *Bd*1 and *Bd*2) (see also Vautrin & Kriebel, 1991).

Most importantly, complex rising phases were often similar in both neurones (Fig. 4*Bd*1 and *Bd*2), suggesting that they were not a characteristic of the postsynaptic detection of the transmitter packet, nor signal attenuation by cable properties of neurites. A very significant attenuation would be required to extend the rise time beyond several milliseconds. Furthermore, such an attenuation should also reduce peak amplitude, but the synaptic events with the most complex rising phases and longest overall rising phases were far from being the smallest. It is unlikely that pairs-recording samples neurones whose synaptic contacts are consistently at matching electrotonic distances to both cell somas. Rather, because the complex rising phases were often similar, we can conclude that these rising phases reflect the actual time course of the GABA release.

The origin of the interdependency between the subunits composing complex TTX-resistant synaptic currents is not clear. These interdependencies could be supported by TTX- and QX-314-resistant Na^+ or Ca^{2+} conductances that would not propagate to the soma and, thus, would go undetected. However, the most convincing evidence that coincident synaptic currents do not result from the synchronized release of transmitter from different presynaptic sites evoked by undetected Na^+ and/or Ca^{2+} currents lies in the observation that the rising phase and frequently its slope changes coincided in the pair. Thus, the most elementary transmissions detected postsynaptically often coincided in these cultured hippocampal neurones.

The complex rising phases did not always match (Fig. 4*Ba*1, *Ba*2, *Bf*1 and *Bf*2). If the sudden increases in rising slope representing subunitary releases generally coincided, the relative amplitude of the subunitary responses varied within the same miniature signal, implying that transmitter release was not always restricted to a point, but could spread laterally to engage receptors on pre- and postsynaptic membranes which did not overlap exactly.

The prospect of cis-mission is consistent with the morphological and physiological results demonstrating the existence of symmetrical synapses, as reviewed above, and with the detection by Dan & Poo (1992) of fast cholinergic synaptic-like transients in myocytes immediately after injecting acetylcholine intracellularly. Myocytes have no processes, suggesting that, as described in cells forming symmetrical synapses and proposed here for hippocampal neurones, the myocyte membrane must fulfil both pre- and postsynaptic functions at virtually the same location to trigger fast synaptic-like signals. Surprisingly, these embryonic hippocampal neurones exhibit diffuse GABA-IR, suggesting that, like exogenous acetylcholine in myocytes (Dan & Poo, 1992), GABA need not be concentrated at terminal sites to support pulsatile release.

GABAergic cis-mission may be widespread *in vivo*

Although evidence of fast cis-mission in cell culture does not address its physiological relevance *in vivo*, it is clear that hippocampal neurones have the potentiality to convey cis-mission. Furthermore, recurrent inhibition, mediated by either auto-transmission, possibly cis-mission, is functional in neuronal cultures, leading to momentary and complete failure of evoked GABAergic transmission in well-differentiated neurones (Benveniste *et al.* 1989; cf. Wilcox & Dichter, 1994). The presence of presynaptic $GABA_A$ receptors in the nervous system (Richards *et al.* 1987; Floran *et al.* 1988; Peng & Frank, 1989; Zhang & Jackson, 1993) suggests that cis-mission may exist *in vivo* as a form of immediate presynaptic signalling in the absence of any collateral or interneurone. The functional consequence of such self-signalling in GABAergic neurones will depend on the Cl^- ion gradient. If the equilibrium potential for Cl^- ions (E_{Cl}) is less negative than the threshold for voltage-dependent Na^+ and/or Ca^{2+} conductances, GABAergic cis-mission would be excitatory, if not regenerative. If E_{Cl} is more negative than these voltage-dependent conductances, then cis-mission would function as a form of immediate presynaptic inhibition.

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