Supplementary Methods

The care and experimental manipulation of the animals was carried out in accordance with institutional and national guidelines. The internal solution contained (in mM): K-methanesulphonate 133, KCl 7, HEPES 10, Mg-ATP 2, Na_2ATP 2, Na_2GTP 0.5, EGTA 0.05, pH 7.2. Biocytin (0.5%) was added for subsequent morphological reconstruction. The external solution for *in vitro* recordings contained (in mM): NaCl 125, $NaHCO_3$ 25, glucose 25, KCl 2.5, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgCl_2$ 1 (pH 7.3 when bubbled with 95% O_2 and 5% CO_2). Seal resistance was always > 3 GΩ before break-in. The series resistance for *in vitro* and *in vivo* granule cell recordings was 32 ± 3 MΩ (n = 44) and 33 ± 2 MΩ (n = 14), respectively. For mossy fibre terminal recordings these values were 35 ± 10 MΩ (n = 8) and 72 ± 20 MΩ (n = 4), determined from the passive current response to a voltage step at the end of the recording (see Supplementary Fig. 1). Capacitive currents recorded from mossy fibre terminals in response to voltage steps were described by a double-exponential function (Supplementary Fig. 1), with the dominant fast component presumably corresponding to the charging of the terminal, and the slower component representing the axon (Geiger and Jonas, 2000). The fast component was used to estimate the capacitance of the terminal.

Data were filtered at 3 - 10 kHz and acquired at 50 kHz using Axograph 4.8 or 4.9 software (Axon Instruments) and an ITC-18 interface (Instrutech). Resting membrane potentials (V_m) were measured immediately after formation of the whole-cell configuration ('break-in'). Data analysis was carried out using Axograph and Igor Pro (Wavemetrics). Input resistance was calculated from the voltage response to symmetrical hyperpolarising or depolarising current steps (400 ms; 10 - 40 pA) from rest, measured at the end of the step. Sag ratio was calculated as the peak divided by the steady-state input resistance from hyperpolarizing voltage deflections reaching -100 mV. Spike broadening *in vivo* was calculated by normalizing the half-width of the spike following the shortest ISI in the sensory-evoked burst by the half-width of the first spike. Spike

broadening *in vitro* was measured using current-injection evoked spike trains (5 at 100 Hz), and the half-width of the 5th spike was normalized by the half-width of the first spike. EPSC traces were digitally low-pass filtered (3 kHz). The latency of sensory-evoked presynaptic action potentials and postsynaptic EPSCs were corrected to account for the temporal delay of the air puff associated with the application system. For stimulus-evoked EPSCs, synaptic responses were categorized as successes when their amplitudes were larger than a threshold (typically ~7 pA), defined as 4.5-fold the standard deviation of the baseline noise. Connections exhibiting frequent failures in the first EPSC in the train were not included in the analysis. Synaptic charge measurements were made using averaged traces where artefacts were removed by linear interpolation between points immediately before and after the artefacts. EPSCs were defined as slow-rising if the 20-80% rise time was shorter than 1.6-fold of the median of the rise time distribution obtained from the same cell. This cut-off value roughly corresponded to the mean plus four times the standard deviation because the coefficient of variation of the rise time distribution was typically 0.15 in cells that did not show obvious slow-rising events. These criteria corresponded well with events categorized by eye. Statistical comparisons were made using Student's two-sided, paired *t*-test unless otherwise indicated.

The effect of synaptic failures on *in vivo* sensory-evoked EPSCs was evaluated by numerical simulation using the sensory-evoked spiking pattern of *in vivo* mossy fibre boutons (Figure 2) and the average failure rate estimated from mossy fibre stimulation experiments (Figure 4b; 1.2mM $[Ca^{2+}]_e$ *in vitro*). For each presynaptic action potential, a failure rate was defined using a value corresponding to the nearest inter-spike interval shown in Figure 4b. Values from 1000 random trials were averaged for each cell. As described in the Results and in Figure 2, the number, the minimum interval and the mean interval of sensory-evoked presynaptic spikes in single boutons were 4.2 ± 0.6, 1.9 ± 0.5 ms and 12.4 ± 1.7 ms, respectively (n = 3). If there are no failures and there is perfectly reliable transmission across the synapse, then the corresponding values for

postsynaptic sensory-evoked EPSCs should be identical (assuming only one bouton is involved). Taking into account the failure rate, the number, the minimum interval and the mean interval of EPSCs were predicted to be 3.9 ± 0.5, 1.9 ± 0.5 ms and 13.0 ± 1.7 ms, respectively (n = 3). Those were similar to the number (5.4 ± 0.7; P = 0.35), the minimum interval (3.1 ± 0.5 ms; P = 0.27), and the mean interval (13.5 ± 1.0 ms; P = 0.82) of actual sensory-evoked EPSCs (n = 14, unpaired t-test). This result is consistent with our conclusion that the sensory-evoked EPSCs arise from activity in a single mossy fibre.

Reference

Geiger JR, Jonas P (2000) Dynamic control of presynaptic Ca^{2+} inflow by fast-inactivating K^{+} channels in hippocampal mossy fiber boutons. Neuron 28:927-939.