Supplementary Figure 1. Estimation of the size of the readily releasable pool and of the vesicular release probability.

(a) Quantal content of the compound AGCs during the train, $M_t$, plotted versus cumulative quantal content for the recording of a train of AGCs as shown in Fig. 3a. Blue line illustrates a linear fit to the first three points and was used to estimate $N_{ves}$ (abscissa intersection), the number of vesicles being available for release before onset of stimulation. In this cell $N_{ves}$ amounts to 224. The ratio $M_1 / N_{ves}$ then approximates $p_{ves}$ the probability for a given vesicle (out of the pool $N_{ves}$) to be released after (the first) action potential and amounted in this cell to 0.2. After correcting for the number of axons stimulated (calculated as $N_{axons} = M_1 / m = 44.7 / 0.355 = 126$, $m$ is the quantal content of a unitary axon-OPC connection, see results) we arrive at an estimate of the releasable pool per axon of $1.8 (= 224 / 126)$ vesicles in this cell. The dark grey line, the dashed blue line and the light grey line denote how the fit to the first three points would look if $n_{ves}$ equal 1, 2 and 3 vesicles, respectively. We repeated this analysis in 6 OPCs and approximate that each axons contains a pool of $2.8 \pm 0.3$ vesicles ($n_{ves}$) which are readily releasable on a neighboring OPC and which are released with a vesicular release probability of $0.13\pm0.01$ ($p_{ves}$). Red line represents an exponential fit, which was used to estimate the steady state $M_i$ (indicated by the dashed line) and which substantiates the linear fit (blue line) to the early release events. The correctness of the parameters derived from this analysis depends on the stability of the quantal response during the train (see below) and on the assumption that the vesicular release probability is constant during the initial part of the train.

(b) Whether the quantal amplitude changes during the train can be assessed by analyzing asynchronous AGCs which transiently occur after the train of stimulations (inset). This approach is based on the general assumption that asynchronous AGCs are due to spontaneous release of vesicles triggered by the residual calcium signal remaining for several hundred milliseconds following stimulation. This residual calcium signal in callosal axons can be seen in Fig. 5c-e. If quantal amplitude were decreased during the train, e.g. by desensitization of glutamate receptors, then the amplitude of asynchronous AGCs shortly after the train should be smaller than the quantal amplitude (4 pA, see results) and it should recover to the quantal amplitude in the following time. The main panel depicts the mean peak amplitude of asynchronous AGCs versus time. $t=0$ indicates end of the action potential train (200 stimuli @ 50 Hz). To amplify any reductions in quantal amplitude by
tetanic stimulation we applied 200 instead of only 20 stimuli. Asynchronous events (114±12 events per cell) were collected during the first 400 ms after the end of the trains (≥ 10 ms after the last stimulus). The AGC amplitudes and the time of the events peak in each trace were analyzed using the sliding template algorithm (see Methods). For each cell (n=3) the peak amplitudes of asynchronous AGCs were averaged within non-overlapping 10 ms time intervals. The mean values were then averaged across the three cells and plotted versus the corresponding post-train time. No evidence for a decrease of asynchronous AGCs amplitudes followed by a recovery could be detected suggesting that there are no postsynaptic confounding factors of the quantal amplitude during the train. Moreover, the mean amplitude of asynchronous AGCs after 20 stimuli (4.4±0.4 pA, n=4) and after 200 stimuli (4.7±0.4 pA, n=3) both are comparable to the amplitude of miniature AGCs (4±0.2 pA). Inset: Original recording taken from the period immediately after the train (200 stimuli @ 50 Hz) which shows asynchronously occurring AGCs. Note the transiently high frequency of asynchronous events during the first 800 ms after the train. Asterisk indicates the last three action potentials of the train. Scale bar 5 pA, 100 ms.

It should be noted that situations are conceivable in which some of the asynchronous AGCs are not necessarily uni-quantal. Such a situation could arise if the train stimulation induces action potential activity of neurons contacted by callosal axons and if this activity outlasts train stimulation. Those neurons may send back axons through corpus callosum and these axons may also release transmitter on the recorded OPC. In such a case a decrease in quantal amplitude could be obscured by simultaneous release of several vesicles. Then the true \( p_{ves} \) would be smaller and the true \( n_{ves} \) would be larger than reported above.

Reference List