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Heterosynaptic plasticity induced by intracellular tetanization in layer 2/3 pyramidal neurons in rat auditory cortex

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Key points summary

- Learning systems equipped with Hebbian-type associative plasticity are prone to runaway dynamics of synaptic weights and lack mechanisms for synaptic competition; these problems can be resolved by heterosynaptic plasticity: changes at synapses which were not active during the induction.
- We show that in layer 2/3 pyramidal neurons from auditory cortex a purely postsynaptic challenge, intracellular tetanization, can induce heterosynaptic plasticity; similar to visual cortex, plasticity direction depends on initial properties of synapses: inputs with initially low release probability tend to potentiate, while those with initially high release probability tend to depress.
- Induction of heterosynaptic plasticity requires intracellular Ca^{2+} rise and its maintenance involves presynaptic changes, which depend on the nitric oxide signalling pathway.
- We conclude that heterosynaptic plasticity is a common property of supragranular pyramidal neurons mediating cortico-cortical connections in both auditory and visual cortices; it may serve as a mechanism of synaptic weight normalization and synaptic competition in these cortical regions.

Abstract Associative Hebbian-type synaptic plasticity underlies the mechanisms of learning and memory; however, Hebbian learning rules lead to runaway dynamics of synaptic weights and lack mechanisms for synaptic competition. Heterosynaptic plasticity may solve these problems by complementing plasticity at synapses that were active during the induction, with opposite-sign changes at non-activated synapses. In visual cortex, a potential candidate mechanism for normalization is plasticity induced by a purely postsynaptic protocol, intracellular tetanization. Here we asked if intracellular tetanization can induce long-term plasticity in auditory cortex. We recorded excitatory postsynaptic potentials (EPSPs) of regular ($n = 76$) and all-or-none ($n = 24$) type in layer 2/3 pyramidal cells in slices from rat auditory cortex. After intracellular tetanization, 32 of 76 regular inputs (42%) showed long-term depression, 21 inputs (28%) showed potentiation and 23 inputs (30%) did not change. The direction of plasticity correlated with the initial release probability: inputs with initially low release probability tended to be potentiated, while inputs with high release probability tended to be depressed. Thus, intracellular tetanization had a normalizing effect on synaptic efficacy. Induction of plasticity by intracellular tetanization required a rise of intracellular $[Ca^{2+}]$, because it was impaired by chelating intracellular calcium with EGTA. The long-term changes induced by intracellular tetanization involved both pre- and postsynaptic mechanisms. EPSP amplitude changes were correlated with changes of release indices: paired-pulse ratio and the inverse of the coefficient of variation (CV^{-2}). Furthermore,

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at some all-or-none synapses, changes of averaged response amplitude were correlated with a change of the failure rate, without a change of the synaptic potency, measured as averaged amplitude of successful responses. Presynaptic components of plastic changes were abolished in experiments with blockade of NO-synthesis and spread, indicating involvement of NO signalling. These results demonstrate that the ability of purely postsynaptic challenges to induce plasticity is a general property of pyramidal neurons of both auditory and visual cortices.

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Abbreviations CV^{-2}, inverse of the coefficient of variation; LTD, long-term depression; LTP, long-term potentiation; PPR, paired-pulse ratio.

Introduction

Synaptic plasticity is believed to underlie the mechanisms for learning and memory. Because the rules for associative synaptic plasticity correspond to both the temporal contiguity requirement in the classical conditioning paradigm and Hebbian learning, associative plasticity is considered to be the synaptic basis of learning (Malenka & Bear, 2004; Massey & Bashir, 2007; Feldman, 2009). However, a learning system equipped with synapses changing according to unrestrained Hebbian-type rules has two major drawbacks. First, it is prone to runaway dynamics due to a positive feedback intrinsic to the Hebbian learning rule: potentiated synapses have a higher probability of evoking spikes, and thus a higher probability of being further potentiated. Second, such a system lacks mechanisms of synaptic competition which are necessary for synapse selection (Miller, 1996). These two features are essential for the formation of representation maps during development, such as ocular dominance and orientation preference maps in the visual system (Wiesel & Hubel, 1963; Thompson *et al.* 1983), or tonotopic maps in the auditory system (Aitkin *et al.* 1970; Merzenich *et al.* 1975).

The propensity for runaway dynamics can be counteracted by precisely balancing local learning rules for potentiation and depression, such as the duration of time windows in which spike-timing-dependent plasticity leads to potentiation and depression, or differential dependence of potentiation and depression on the initial weight of the synapse (e.g. Song *et al.* 2000; Song & Abbott, 2001; Sjöström *et al.* 2008). Another example of a local balancing mechanism is distance-dependent heterosynaptic plasticity, which surrounds the location at which long-term potentiation (LTP) or long-term depression (LTD) was induced, but has an opposite sign and a magnitude decaying with distance (Lynch *et al.* 1977; White *et al.* 1990; Scanziani *et al.* 1996; Royer & Paré, 2003). Both problems can also be solved by normalization mechanisms which conserve the total synaptic weight of all synapses at a neuron – the solution widely used since the early models (von der Malsburg, 1973). Our previous work in the visual cortex showed that plasticity at synapses to layer 2/3 pyramids can be induced by a purely postsynaptic protocol, intracellular tetanization, which simulates induction of heterosynaptic plasticity at synaptic inputs (Volgushev *et al.* 1994, 1997, 2000a). The direction and magnitude of plasticity induced by intracellular tetanization depend on initial properties of synapses: synapses with initially low release probability are more susceptible for potentiation, while synapses with initially high release probability have a tendency to be depressed or not to change (Volgushev *et al.* 1997, 2000a). Similar dependence was reported in visual cortical neurons following pairing of monosynaptic input with strong postsynaptic firing (Hardingham *et al.* 2007; Sáez & Friedlander, 2009). Since this dependence was observed at synapses which were either stimulated (Hardingham *et al.* 2007; Sáez & Friedlander, 2009) or not stimulated (Volgushev *et al.* 2000a) during the plasticity induction, it may represent a cell-wide mechanism for synaptic weight normalization (Chistiakova & Volgushev, 2009). While this phenomenon has been observed in visual cortex neurons, it is unclear whether it is a unique feature of the visual cortex, or if it may represent a more general homeostatic mechanism of regulation of synaptic efficacy in neurons across other cortical areas. To address this question, it is necessary to study plasticity in different cortical regions.

In auditory cortex, tonotopic representation of sounds is formed during development and is experience dependent (Buonomano & Merzenich, 1998; Weinberger, 2004), suggesting involvement of synaptic plasticity in this process. Field potential studies showed that LTP in auditory cortex can be induced by high frequency afferent stimulation (Kudoh & Shibuki, 1994, Seki *et al.* 2001), and correlated activity (Ahissar *et al.* 1992; Kudoh & Shibuki, 1997). LTD has been observed following low frequency afferent stimulation (Kudoh *et al.* 2002), and high frequency local stimulation (Watanabe *et al.* 2007). Intracellular study revealed that pairing-induced plasticity in the auditory cortex is NMDA-receptor dependent (Buonomano, 1999). Here we asked (1) whether a purely

postsynaptic protocol can induce plasticity in rat auditory cortex neurons, (2) whether plasticity induction depends on initial properties of synapses, and (3) whether presynaptic or postsynaptic mechanisms are involved in maintenance of the plastic changes.

Methods

Slice preparation

All experimental procedures used in this study were in compliance with the US National Institutes of Health regulations and were approved by the Institutional Animal Care and Use Committee of the University of Connecticut. Details of slice preparation and recording were similar to those used in previous studies (Volgushev *et al.* 2000a,b). Wistar rats (15–32 days old) were anaesthetized with isofluorane, decapitated, and the brain was quickly removed and placed into an ice-cold oxygenated artificial cerebrospinal fluid solution (ACSF) containing, in mM: 125 NaCl, 25 $NaHCO_3$, 25 glucose, 3 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, bubbled with 95% O_2–5% CO_2, pH 7.4. Coronal slices (350 μm thickness) containing auditory cortex were prepared from the right hemisphere. Slices were allowed to recover for at least an hour at room temperature. For recording, individual slices were transferred to a recording chamber mounted on an Olympus microscope equipped with infrared differential interference contrast (IR-DIC) optics. In the recording chamber slices were submerged in oxygenated ACSF at 30–32°C. Layer 2/3 pyramidal cells from auditory cortex were selected for recording in the whole cell configuration. The intracellular pipette solution contained, in mM: 130 potassium glutamate, 20 KCl, 10 Hepes, 10 sodium phosphocreatine, 4 Mg-ATP, 0.3 Na_2-GTP (pH 7.4 with KOH). In experiments with intracellular calcium chelation, calcium chelator ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA, 10 mM) was added to the intracellular solution. In a separate series of experiments the inhibitor of NO synthase, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME, 20 μM), and the NO scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, potassium salt (carboxy-PTIO, 20 μM) were added to the bath medium.

Intracellular recording and synaptic stimulation

Two pairs of stimulating electrodes (S1 and S2) were placed in layer 4, below the layer 2/3 recording site (Fig. 1*A*). Stimulation current intensities were adjusted to evoke monosynaptic excitatory postsynaptic potentials (EPSPs) in the layer 2/3 cell. Identification of pyramidal neurons using DIC microscopy was reliable as demonstrated in our previous work with biocytin labelling and morphological reconstruction of recorded neurons (Volgushev *et al.* 2000a,b). We used a paired-pulse stimulation protocol with a 50 ms inter-pulse interval. Paired stimuli were applied to S1 and S2 in alternating sequence once per 7.5 s, so that each input was stimulated with paired pulses each 15 s. EPSPs in the layer 2/3 cell induced by layer 4 stimulation were recorded during a 10–12 min control period. Following the control period, synaptic stimulation was stopped and intracellular tetanization was applied to the layer 2/3 cell through the recording pipette. Intracellular tetanization consisted of three trains (1/min) of ten bursts (1 Hz) of five pulses (5 ms, 100 Hz, 0.4–1.1 nA, Fig. 1*B*). The current intensity was adjusted to evoke 4–5 spikes per burst. In the majority (35 of 55) of cells, each pulse in a train evoked a spike. Following intracellular tetanization, synaptic stimulation was resumed, and EPSPs evoked by the test stimuli were recorded for another 40–50 min.

Data analysis

All inputs included in the analysis fulfilled the criteria of (1) stability of EPSP amplitudes during the control period, (2) stability of the membrane potential throughout the recording, and (3) stability of the onset latency and kinetics of the rising slope of the EPSP. EPSP amplitudes were measured as the difference between the mean membrane potential during two time windows. The first time window was placed before the EPSP onset and the second time window was placed just before the peak of the rising slope of the EPSP (Fig. 1*C* and *D*, grey bars). The amplitude of the second EPSP in the paired-pulse stimulation protocol was measured using windows of the same duration, but shifted by the length of the inter-pulse interval (50 ms).

For assessing changes of synaptic efficacy, the amplitude of EPSPs evoked by the first stimulus in a pair was used. The magnitude of plastic changes was calculated as the ratio of average EPSP amplitude after the tetanization over the average EPSP amplitude during the control period. For calculation of averaged post-tetanic response amplitude, 30–190 stable responses (period of 7.5–47.5 min, starting 2–42 min after the tetanization) were used. For calculation of the EPSP amplitude in control, 10–45 responses during the control period were used. Examples of control and post-tetanic time periods used for calculation of averaged responses are shown as red and blue bars above the time course of EPSP amplitude changes in Fig. 2.

The criterion for plasticity was a significant ($P < 0.05$, Student's *t* test) change in mean EPSP amplitude between the control and post-tetanic time periods. For both control and post-tetanic periods, the slope of the EPSP, paired-pulse ratio (PPR) and inverse of the coefficient of variation (CV^{-2}) were calculated. To measure EPSP slope,

a cubic polynomial function was used to fit the rising slope of EPSP. The value of the slope was determined by evaluating the derivative of the polynomial at half of the interval between the onset and the maximum of the EPSP. PPR was calculated as the ratio of the averaged amplitude of the EPSP evoked by the second pulse in the paired-pulse protocol over the averaged amplitude of the EPSP evoked by the first pulse. The inverse of the coefficient of variation (CV^{-2}) was calculated for the EPSPs evoked by the first stimulus in a pair, as $(Mean)^2/(Variance)$, with response variance corrected by the variance of the noise.

Results

The effect of intracellular tetanization on synaptic efficacy was tested at 100 synaptic inputs to 55 pyramidal cells from layer 2/3 in rat auditory cortex (Fig. 1*A*). Two types of synaptic responses were observed. At a majority of synapses, 76 out of 100, EPSPs were of 'regular' type, with a gap-free distribution of response amplitudes, and variance of the response amplitude higher than the variance of the noise (Fig. 1*C*). At the remaining 24 inputs (24%), EPSPs were of all-or-none type, characterized by large amplitude but small amplitude variance (Fig. 1*D*). These all-or-none responses were similar to those described earlier in slices from the visual cortex of rats (Volgushev *et al.* 1995) and cats (Stratford *et al.* 1996). Because of their distinct properties, the regular and the all-or-none responses will be considered separately.

Intracellular tetanization may induce LTP or LTD, or does not change synaptic efficacy

Intracellular tetanization induced long-term plasticity at the majority of regular inputs (53 out of 76, or 69.7%). Of the regular inputs, 21 (27.6%) underwent LTP, 32 (42.1%) underwent LTD, and 23 inputs (30.3%) did not change after intracellular tetanization (Fig. 2).

Figure 2*A* shows an example of long-term potentiation of synaptic responses induced by intracellular tetanization. The top panel in Fig. 2*A* shows an overlay of averaged responses recorded during the control period before

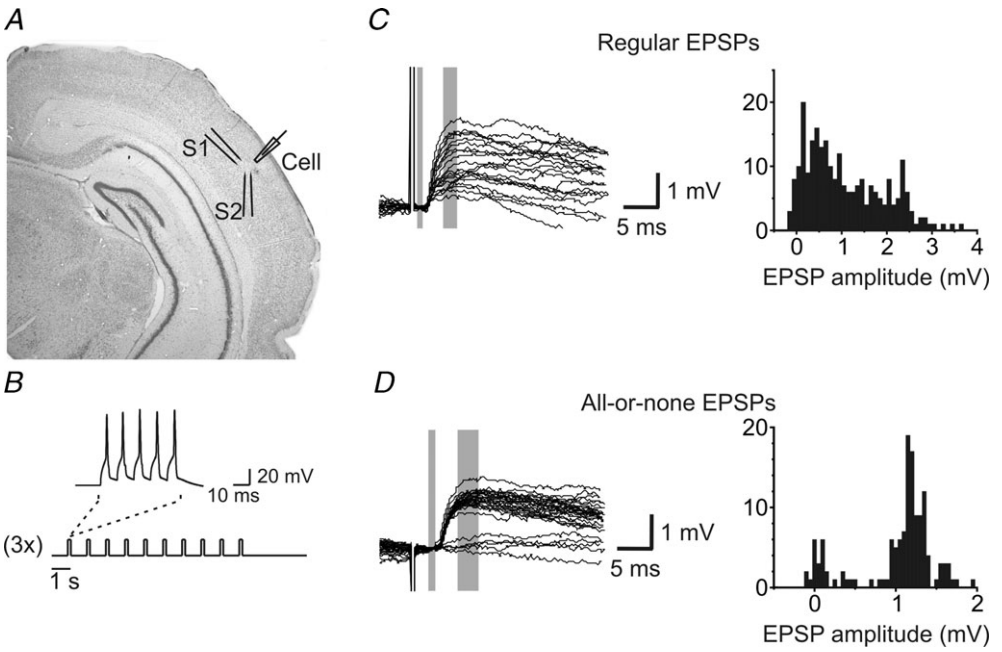

Figure 1. Two types of excitatory postsynaptic potentials in layer 2/3 cells in auditory cortex: regular and all-or-none responses
A, a scheme of the location of the recording and stimulation electrodes in the auditory cortex in a slice. Recordings were made from layer 2/3 pyramidal neurons. Two pairs of stimulation electrodes (S1 and S2) were placed in layer 4, below the electrode for intracellular recording. *B*, illustration of the intracellular tetanization protocol. Intracellular tetanization consisted of 3 trains (one per minute) of 10 bursts (one per second) of 5 short depolarizing pulses (5 ms pulse duration, 100 Hz) applied through the recording electrode. Each burst of five pulses evoked 4–5 spikes (top trace). *C,* example of regular EPSP. Superposition of 20 individual responses (left) and their amplitude distribution (right). Grey boxes (left) show the windows used for amplitude measurements. Note large variability of the response amplitude and a gap-free amplitude distribution. *D,* example of an all-or-none EPSP, conventions as in *C*. Note that electrical stimulation evoked either a clear response with a large, uniform amplitude or a failure. This results in a clearly bimodal amplitude distribution, with variability of the response amplitude similar to the variability of the noise (failures).

tetanization (blue trace and blue bar above the time course of response amplitude changes) and during the post-tetanic period 30–50 min after the tetanization (red trace and red bar above the time course). In this cell the mean EPSP amplitude during the post-tetanic period was significantly larger (167.8%, $P < 0.05$) than the mean EPSP amplitude in control. Intracellular tetanization led to a significant ($P < 0.05$) potentiation of EPSP amplitude in 27.6% (21 out of 76) synaptic inputs. Figure 2*D* shows the time course of EPSP amplitude changes, averaged over all 21 potentiated inputs. Potentiation of the response amplitudes built up during the first 20 min following tetanization, then plateaued. For the 21 inputs which expressed significant potentiation after the intracellular tetanization, the mean EPSP amplitude increased to $168.1 \pm 11.0\%$ ($n = 21$) of the response amplitude in control.

Figure 2*B* shows an example of long-term depression of synaptic responses induced by intracellular tetanization. In this cell the mean EPSP amplitude after the tetanization was significantly smaller (64.1%, $P < 0.05$) than the mean EPSP amplitude during the control period. Significant decrease of the response amplitude after intracellular tetanization was observed in 42.1% (32 out of 76) inputs. In the 32 depressed inputs, EPSP amplitudes gradually decreased during the first 35 min after the tetanization, then stabilized (Fig. 2*E*). On average, post-tetanic EPSP amplitudes decreased to $62.6 \pm 3.0\%$ ($n = 32$) of the control response amplitudes.

The remaining 23 of 76 regular inputs (30.3%) did not change after intracellular tetanization. Figure 2*C* shows an example of synaptic responses which did not show plasticity. The mean post-tetanic EPSP amplitude was 93.6% of the control EPSP amplitude. This difference was not significant ($P > 0.05$). For the 23 inputs that did not show plasticity, EPSP amplitudes were stable throughout the testing period (Fig. 2*F*), and the mean post-tetanic response amplitude was $99.6 \pm 3.2\%$ ($n = 23$) of the control.

When all 76 regular inputs were pooled together, the averaged EPSP amplitude showed some increase during the first 30 min following tetanization, but then returned

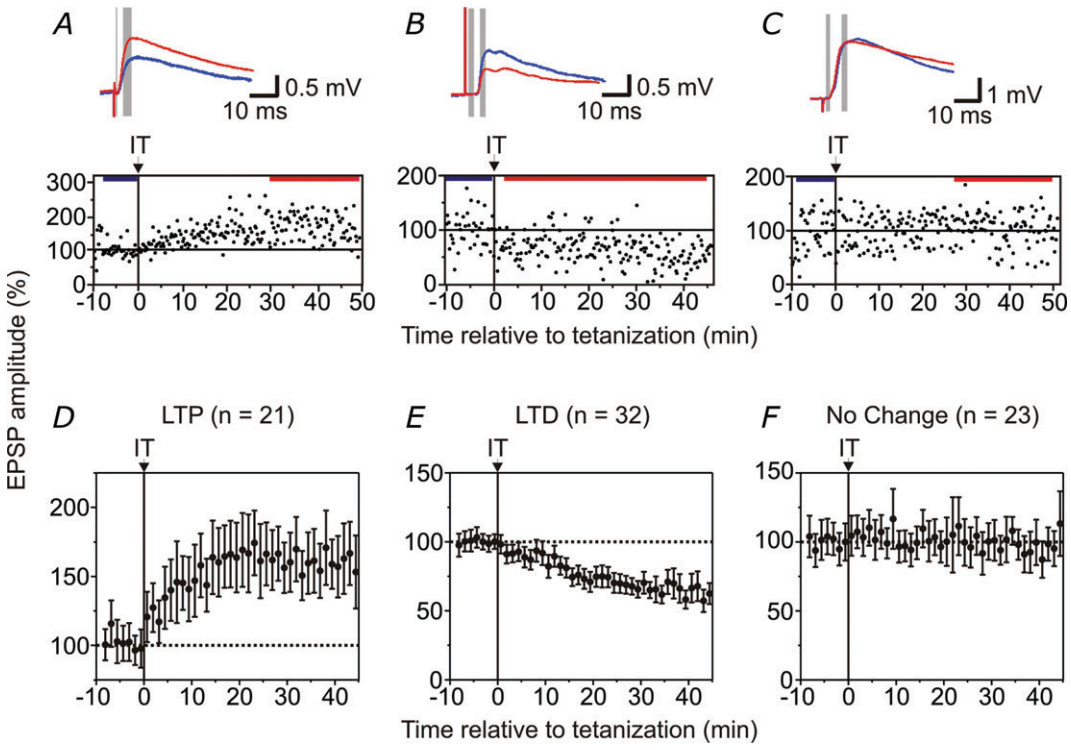

Figure 2. Long-term changes of synaptic transmission induced by intracellular tetanization
A–C, example of inputs that expressed long-term potentiation (*A*), long-term depression (*B*), or did not change after intracellular tetanization (*C*). EPSP amplitudes were measured as a difference between mean potential in two windows, shown as grey boxes on top of each pair of traces. The blue trace shows the averaged EPSP before the tetanization, and the red trace, after the tetanization. Pre-tetanic and post-tetanic time intervals from which averaged EPSPs were calculated are indicated as the blue and the red bars above the time course of EPSP amplitude changes. In the time courses, EPSP amplitude changes are shown relative to the average EPSP amplitude before the tetanization. The vertical line indicates the timing of intracellular tetanization. *D–F*, time course of EPSP amplitude changes in inputs expressing LTP (*D*, $n = 21$), LTD (*E*, $n = 32$) or no change after the intracellular tetanization (*F*, $n = 3$).

to the baseline. The transient potentiation during the early post-tetanic period may be explained by the slower build-up of LTD compared to the LTP (Fig. 2*D* and *E*). After about 30 min, when both potentiation and depression levelled off, there was no net long-term change of EPSP amplitudes after intracellular tetanization. These results suggest that intracellular tetanization does not have a general long-term potentiating or depressing effect across all synapses of the postsynaptic cell.

Thus, robust synaptic plasticity in both directions, LTP or LTD, can be induced by intracellular tetanization at individual synaptic inputs to layer 2/3 pyramidal neurons in the auditory cortex. Both potentiation and depression developed slowly over 20–30 min following the tetanization, and stabilized ∼30 min after tetanization.

Plasticity induction by intracellular tetanization is not generalized across a cell but depends on input properties

Intracellular tetanization may lead to potentiation or depression or no change of synaptic efficacy. We asked, if susceptibility for potentiation or depression is (a) a property of a neuron, so that some cells express potentiation but other cells express depression, or, alternatively, (b) a property of an input, so that some inputs to the same cell may express potentiation but some other inputs express depression?

To distinguish between these two possibilities, we first examined if plastic changes at two inputs to the same cell are correlated. In 27 of the 55 recorded neurons, both simultaneously tested inputs were of regular type and fulfilled the stability criteria. In 14 of these 27 cells, intracellular tetanization induced plasticity at both inputs. In 10 cells one input expressed plasticity while the other did not change, and in 3 cells both inputs did not change. Of the 14 cells showing plasticity at both inputs, in 8 cells the two inputs changed in opposite directions, and in 6 cells both inputs changed in the same direction. Figure 3*A* summarizes these results. There was no correlation between the direction and magnitude of change (or the absence of change) at the two inputs to the same cell after intracellular tetanization. Thus, both the induction of plasticity and the direction of plastic changes were not generalized across all inputs to a cell.

To examine whether variation in the plasticity induction protocol may have contributed to a variation of the synaptic changes, we examined the relationship between the number of spikes evoked in a train during intracellular tetanization, and the resulting EPSP amplitude change (Fig. 3*B*). During intracellular tetanization, the intensity of the pulses was adjusted to evoke 4–5 spikes per burst. During a train, 10 bursts (50 pulses) were applied to the cell, with the number of spikes evoked in each train ranging from 40 to 50. For the majority of inputs (47 of 76), each current pulse evoked an action potential, thus eliciting 50 spikes in a train. For the other inputs, some current pulses failed to induce an action potential. Figure 3*B* shows the relationship between the number of spikes in a train during intracellular tetanization, and the subsequent change of EPSP amplitude. Mean EPSP amplitude change did not vary between inputs grouped by number of spikes per train ($F = 0.115$, n.s.). Inputs at which 43 spikes were evoked during a train showed greater variance of EPSP amplitude change than inputs at which 50 spikes were evoked ($F = 10.94$, $P < 0.01$). However, variance was not different between other groups of inputs. Thus, in our experimental conditions, variability of the magnitude and overall direction of plasticity were not related to the different number of spikes during the tetanization. Even intracellular tetanization with 40 spikes per train (the minimum in our experiments) was sufficient to induce plasticity.

Next we asked if different outcomes of intracellular tetanization could be attributed to the differential distances of investigated synapses from the soma. Recent studies demonstrated that EPSP kinetics, specifically the rising slope, depend on the distance of stimulated synapses from the soma (Letzkus *et al.* 2006; Sjöström & Häusser, 2006). Figure 3*C* shows that there was no significant correlation between changes of EPSP amplitude following intracellular tetanization and the slope of control EPSP ($r = -0.072$, $P = 0.54$, n.s.). In Fig. 3*C*, the majority of EPSPs formed a homogeneous cloud with slopes below 0.6 mV ms^{-1}. Inputs that express potentiation, depression or did not change were represented in this cloud of points, indicating that the majority of the studied EPSPs formed a homogeneous sample, and minor differences in their kinetics could not account for the differential effects of intracellular tetanization.

Previous studies of synaptic plasticity in pyramidal cells from rat visual cortex have revealed that the direction and degree of the plasticity depends on the initial properties of the synapse (Volgushev *et al.* 1997, 2000a; Hardingham *et al.* 2007). These studies showed that synapses with an initially low probability of release were more likely to be potentiated, while synapses with an initially high probability of release were more likely to be depressed. To estimate properties of release at the presynaptic terminal, we used paired-pulse ratio (PPR), which is inversely related to the release probability (for reviews, see Stevens, 1993; Voronin, 1993). At synapses with a low probability of release, PPR is expected to be high. Consistent with previous findings in the visual cortex, changes in EPSP amplitudes were correlated with the initial PPR ($r = 0.384$, $P < 0.001$) measured during the control period (Fig. 4*A*).

To study this relation further, we segregated the regular inputs into two groups, those with initial PPR below the median (PPR < 0.898, mean = 0.676 ± 0.030,

$n = 38$) and those with the initial PPR above the median (PPR > 0.898, mean = 1.399 ± 0.081, $n = 38$). Inputs with initially high release probability (low PPR) did not express a net change of EPSP amplitudes after intracellular tetanization (88.5 ± 6.6%, $P = 0.092$, Fig. 4*B*). In contrast, inputs with initially low release probability (high PPR) expressed a weak net potentiation (117.4 ± 9.2%) after the intracellular tetanization (Fig. 4*C*). Significance of the amplitude change at these inputs ($P = 0.063$) was just below the criterion we used throughout this study, but EPSP amplitude change was significantly greater for inputs with high initial PPR than for inputs with low initial PPR ($P < 0.05$).

Taken together, these results show that the induction of plasticity and the direction of plastic changes were not generalized over all synapses to one cell, but were related to the initial PPR at a particular input and thus regulated individually at each input.

Changes of response amplitude induced by intracellular tetanization are associated with changes of release indices

Because induction of plasticity by intracellular tetanization was related to the initial state of presynaptic release mechanisms, we asked if presynaptic mechanisms are also involved in the maintenance of plastic changes. We have calculated two indices of presynaptic release, PPR and the inverse of the coefficient of variation (CV^{-2}) of responses during the control and during the post-tetanic period, and examined if changes in these release indices

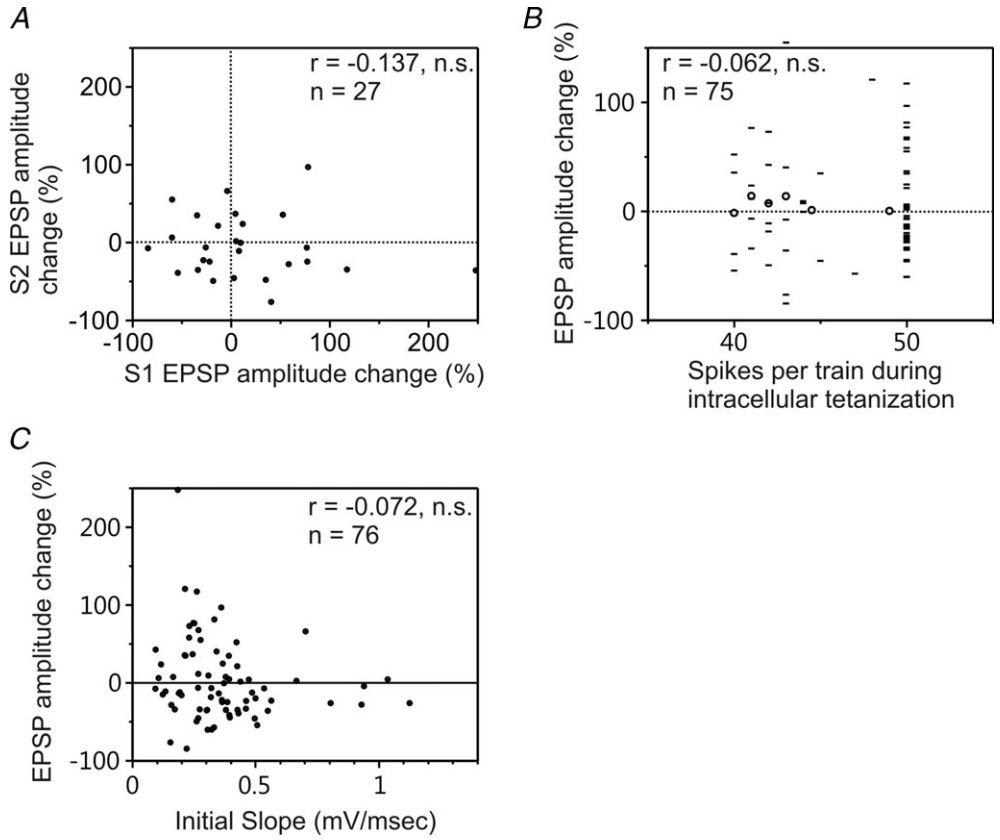

Figure 3. Changes of EPSP amplitude induced by intracellular tetanization were not correlated in two inputs to the same cell and were not related to variation of the number of spikes during the tetanization
A, the plastic changes induced by intracellular tetanization at two inputs to one cell were not correlated ($r = –0.137$, $n = 27$). For those neurons in which two independent inputs were tested, the change of EPSP amplitude at one input is plotted against the EPSP amplitude change at the other input. *B,* changes of EPSP amplitude were not related to the number of spikes evoked during intracellular tetanization ($r = –0.062$, $n = 75$). Changes of EPSP amplitude are plotted against number of spikes per train. Circles show averaged EPSP amplitude change for inputs grouped by number of spikes per train. Cases with 44 and 45, and cases with more than 46 spikes per train were pooled, respectively, due to low sample sizes. For one input, EPSP amplitude change fell outside of the window of the scatter plot (43 spikes in a train during intracellular tetanization, the EPSP amplitude following tetanization 248% of the baseline). *C*, the direction of plasticity was not dependent on the initial slope of the EPSP ($r = –0.072$, $n = 76$). Changes of EPSP amplitude are plotted against initial slope.

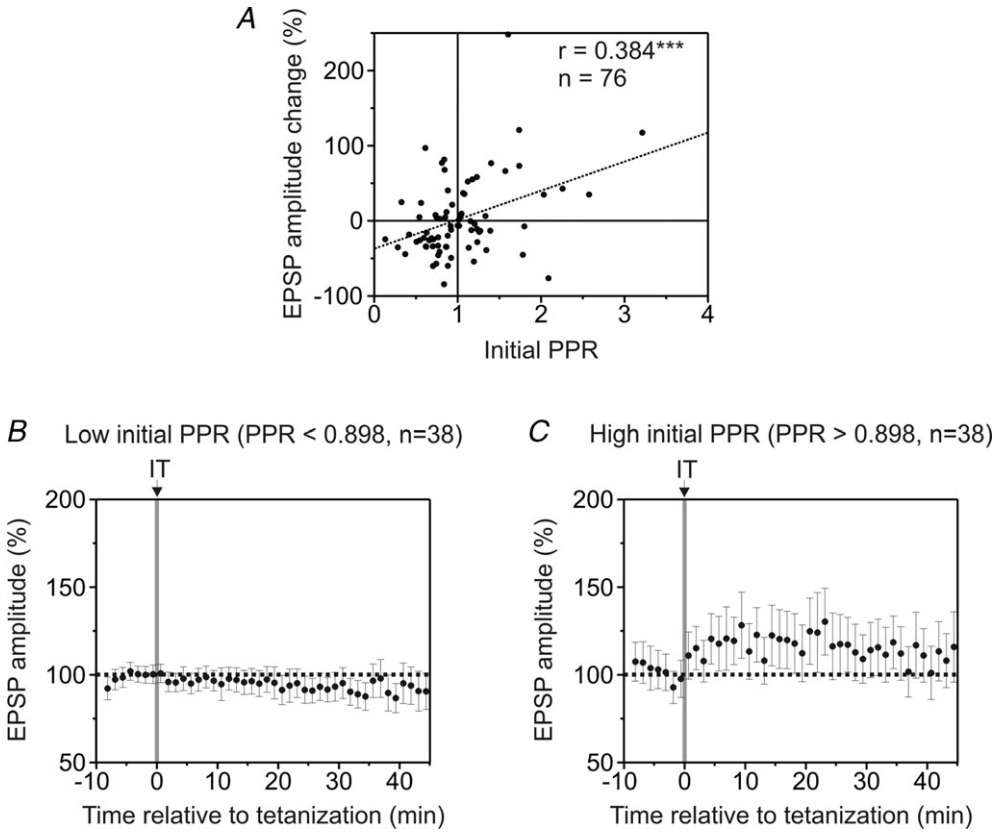

Figure 4. Changes of EPSP amplitude induced by intracellular tetanization are related to the initial paired-pulse ratio
A, changes of EPSP amplitude following intracellular tetanization plotted against the initial paired-pulse ratio ($r = 0.384$, $^{***}P < 0.001$, $n = 76$). *B* and *C,* time course of EPSP amplitude changes after intracellular tetanization in the inputs with initial PPR below or above the median (*B*, PPR < 0.898; *C*, PPR > 0.898).

are associated with the response amplitude changes. Figure 5*A* shows that changes in EPSP amplitudes following intracellular tetanization were significantly negatively correlated with changes of PPR ($r = –0.375$, $P < 0.001$). Since release probability is inversely related to the PPR, this correlation implies that potentiation of EPSP amplitude was associated with an increase of the release probability, and depression was associated with a decrease of the release probability. Changes in EPSP amplitudes following tetanization were positively correlated with changes of CV^{-2} ($r = 0.525$, $P < 0.001$, Fig. 5*B*). CV^{-2} is proportional to release probability, hence

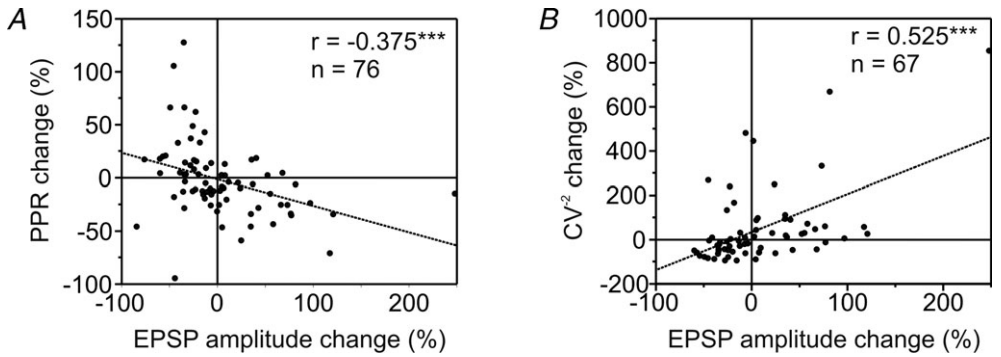

Figure 5. Synaptic plasticity induced by intracellular tetanization is associated with changes of release probability indices
A, correlation of changes of the paired-pulse ratio with changes of EPSP amplitude following intracellular tetanization ($r = –0.375$, $^{***}P < 0.001$, $n = 76$). *B,* correlation of changes of CV^{-2} with changes of EPSP amplitude following intracellular tetanization ($r = 0.525$, $^{***}P < 0.001$, $n = 67$).

significant positive correlation of the EPSP amplitude changes to the CV^{-2} changes substantiates the above conclusion. Thus, both PPR and CV^{-2} results suggest that plastic changes of EPSP amplitude induced by intracellular tetanization were at least partially mediated by presynaptic mechanisms: LTP was associated with an increase in the probability of release, and LTD with a release probability decrease.

However, some inputs expressed significant changes of EPSP amplitude without a change of PPR or CV^{-2}, indicating that plasticity could also occur without observable changes of release probability. These results suggest that intracellular tetanization may activate both pre- and postsynaptic mechanisms supporting plasticity, whereby at a particular synapse pre- or postsynaptic mechanisms may dominate.

Induction of plasticity by intracellular tetanization requires a rise of intracellular calcium concentration

To probe the mechanisms underlying induction of plasticity by intracellular tetanization, we studied the effect of intracellular tetanization on synaptic efficacy using an intracellular solution with 10 mM of the calcium chelator EGTA. Although changes of EPSP amplitudes in both directions were observed following intracellular tetanization (Fig. 6), their magnitude was smaller than in control experiments. The variance of the EPSP amplitude changes in EGTA experiments was significantly reduced compared to control (with EGTA: 427.7, $n = 20$; control: 2755, $n = 76$, $P < 0.05$).

As illustrated in Fig. 6*A*, changes of EPSP amplitude were correlated with initial paired-pulse ratio ($r = 0.504$, $P < 0.05$). The same type of relationship between EPSP amplitude changes and initial paired-pulse ratio was also observed under control conditions. We separated inputs into two groups based on initial PPR relative to the median initial PPR of control inputs (median initial PPR = 0.898). Figure 6*B* and *C* illustrates the time courses of EPSP amplitudes for inputs with a low or high initial PPR in EGTA experiments. EPSPs at inputs with a lower initial PPR expressed a net depression following intracellular tetanization ($83.9 \pm 6.2\%$, $P < 0.05$, $n = 8$). EPSPs at inputs with higher initial PPR did not change amplitude ($99.0 \pm 6.1\%$, $P = 0.87$, $n = 12$). Thus, although EGTA application reduced the degree of plasticity in comparison to controls, dependence of synaptic changes on release properties of the input was preserved, as was observed in control conditions.

Changes of EPSP amplitudes in EGTA experiments were correlated with changes of paired-pulse ratio (Fig. 6*D*, $r = -0.502$, $P < 0.05$, $n = 20$) but correlation with changes of CV^{-2} did not reach significance level (Fig. 6*E*, $r = 0.323$, $P = 0.165$, $n = 20$). The involvement of presynaptic mechanisms in the maintenance of plastic changes induced with reduced levels of intracellular calcium rise is less clear.

Presynaptic changes following intracellular tetanization are partially mediated via nitric oxide signalling pathway

The involvement of presynaptic mechanisms in the maintenance of plastic changes implies retrograde signalling. Previous research suggested involvement of nitric oxide in retrograde signalling in the hippocampus (O'Dell *et al.* 1991) and visual cortex (Volgushev *et al.* 2000a). To explore the possible role of retrograde signalling via the nitric oxide pathway in auditory cortex, we used bath application of the NO synthase inhibitor L-NAME (20 μM) and the NO scavenger carboxy-PTIO (20 μM). In experiments with 20 μM L-NAME and 20 μM carboxy-PTIO in the extracellular medium, we observed changes in the amplitudes of EPSPs in both directions following intracellular tetanization (Fig. 7). However, plasticity induced in these experiments exhibited several important differences from plastic changes in control experiments. First, it was of smaller magnitude. The variance of the EPSP amplitude changes was significantly reduced compared to control (with NO blockade: 870.0, $n = 29$; control: 2755, $n = 76$, $P < 0.05$), indicating that disruption of NO signalling reduced the magnitude of plasticity induced by intracellular tetanization. Second, the correlation between EPSP amplitude changes and initial paired-pulse ratio was abolished (Fig. 7*A*, $r = 0.200$, $n = 29$, $P = 0.30$). Figure 7*B* and *C* shows the time course of averaged EPSP amplitude changes in two groups of inputs, segregated by initial PPR relative to the median initial PPR of control inputs (median initial PPR = 0.898). EPSPs at inputs with a lower initial PPR tended to be depressed after intracellular tetanization ($81.8 \pm 8.6\%$, $P = 0.072$, $n = 8$). EPSPs at inputs with higher initial PPR did not show net change of the amplitude ($98.3 \pm 6.7\%$, $n = 21$). Thus, disruption of NO signalling has also disrupted the dependence of EPSP amplitude changes on initial release properties of the inputs. Finally, the correlation between EPSP amplitude changes and changes of presynaptic release indices, PPR (Fig. 7*D*, $r = -0.221$, $n = 29$), and CV^{-2} (Fig. 7*E*, $r = 0.338$, $n = 28$) was weakened and did not reach significance level in experiments with L-NAME and carboxy-PTIO. Altogether, these results suggest that at least some of the response changes following intracellular tetanization are presynaptic, and a NO signalling pathway is at least partially responsible for the presynaptic changes.

Summary of effects of calcium chelation and disruption of NO signalling on plasticity induced by intracellular tetanization

Figure 8 shows the mean changes of EPSP amplitude following intracellular tetanization under control conditions, intracellular calcium chelation with EGTA application, and disruption of NO signalling by L-NAME and carboxy-PTIO application. Under conditions of both calcium chelation and NO signalling disruption, changes of EPSP amplitude were of smaller magnitude compared to control, and were induced less frequently than in

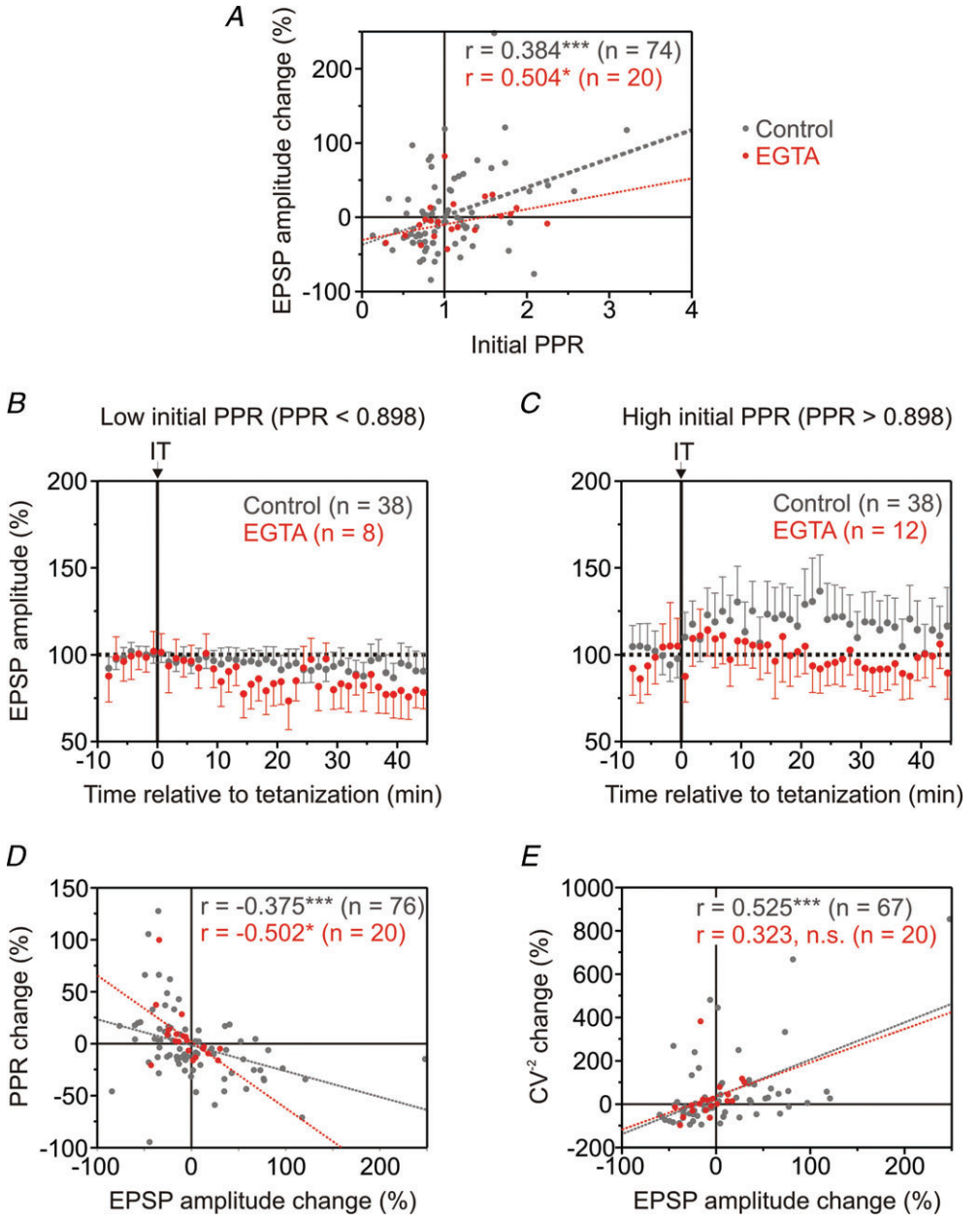

Figure 6. Induction of synaptic plasticity by intracellular tetanization requires a rise of intracellular [Ca^{2+}]

A, changes of EPSP amplitude following intracellular tetanization plotted against initial PPR for experiments with 10 mM EGTA in the recording pipette (red symbols). Note that the magnitude of EPSP changes in EGTA experiments was reduced compared to control. EPSP amplitude changes were correlated with initial paired-pulse ratio ($r = 0.504$, $^*P < 0.05$, $n = 20$). *B* and *C,* time course of EPSP amplitude changes in the inputs with initial PPR above or below 0.898 (median for control experiments). *D* and *E,* EPSP amplitude changes following intracellular tetanization plotted against changes of PPR (*D*, $r = 0.502$, $^*P < 0.05$, $n = 20$) and changes of CV^{-2} (*E*, $r = 0.323$, n.s., $n = 20$). In *A–E*, red symbols show results of experiments with EGTA, and light grey symbols show control data from Fig. 4 for comparison.

control conditions. Variance of EPSP amplitude in either condition was significantly lower than in control ($P < 0.05$ for both comparisons). Significant decrease of plasticity under either condition shows that in control conditions EPSP amplitude changes were indeed due to activation of plasticity mechanisms by intracellular tetanization rather than to instability of responses. Further, there was a general shift of plasticity towards depression (Fig. 8). The clearest evidence of this change was the impairment of the induction of potentiation. Potentiation was induced in fewer cases (4 out of 20, or 20% in EGTA experiments, and 5 out of 29, or 17.2% in experiments

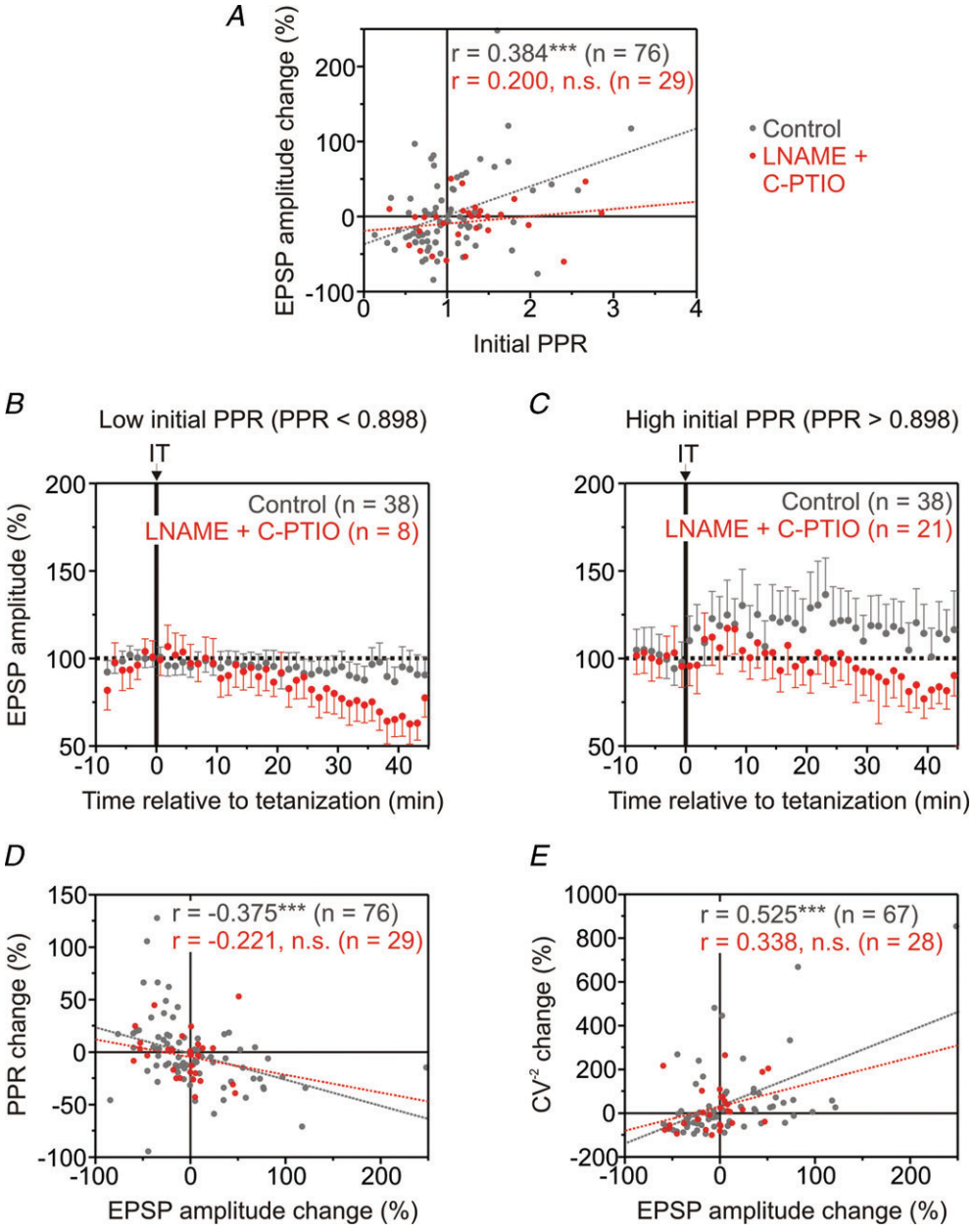

Figure 7. Dependence of EPSP amplitude changes induced by intracellular tetanization on initial release conditions, and presynaptic changes, are abolished by application of L-NAME and carboxy-PTIO
A, changes of EPSP amplitude following intracellular tetanization plotted against initial PPR for experiments with 20 μM L-NAME and 20 μM carboxy-PTIO (C-PTIO) in the extracellular medium (red symbols). Under these conditions, the dependence of EPSP amplitude changes on initial paired-pulse ratio was abolished ($r = 0.200$, $n = 29$). *B* and *C,* time course of EPSP amplitude changes in the inputs with initial PPR above or below 0.898. *D* and *E,* EPSP amplitude changes following intracellular tetanization in the presence of L-NAME and C-PTIO were not correlated with either changes of PPR (*D*, $r = 0.221$, n.s., $n = 29$) or changes of CV^{-2} (*E*, $r = 0.338$, n.s., $n = 28$). In *A–E*, red symbols show results of experiments with 20 μM L-NAME and 20 μM carboxy-PTIO, and light grey symbols show control data from Fig. 4 for comparison.

with disrupted NO signalling, compared to 21 out of 76, or 27.6% in control) and was of a smaller magnitude ($122.2 \pm 4.1\%$ in EGTA experiments, $135.3 \pm 7.8\%$ in experiments with disrupted NO signalling, compared to $168.1 \pm 11.0\%$ in control). Impairment of induction of long-term potentiation is further illustrated by the absence of net changes in inputs with initially high PPR ($99.0 \pm 6.1\%$ in EGTA experiments, and $98.3 \pm 6.7\%$ in experiments with disrupted NO signalling), which in control showed net potentiation ($117.3 \pm 9.2\%$). The proportion of inputs exhibiting depression was also lower (35% in EGTA experiments, 24.1% in experiments with disrupted NO signalling, compared to 42.1% in control). Respectively, higher proportions of inputs did not change after intracellular tetanization in EGTA experiments (45%) and experiments with disrupted NO signalling (58.6%) compared to control (30.3%).

Plasticity of all-or-none synaptic responses

In addition to the 76 regular inputs, the effect of intracellular tetanization was also studied in 24 inputs (24%) exhibiting all-or-none responses. All-or-none responses are characterized by a clearly bimodal amplitude distribution with peaks representing release failures and successful responses clearly separated (Figs 1, 9 and 10). The successful responses have large amplitude and a low variability (see Volgushev *et al.* 1995 and Stratford *et al.* 1996 for all-or-none responses in the visual cortex). For an all-or-none synapse, the number of successful responses and release failures can be counted unambiguously, allowing for direct measurement of the release probability, as a proportion of successful responses, and synaptic potency, which is the amplitude of successful responses. If plasticity is induced at these synapses, presynaptic changes would be expected to change the failure rate, while postsynaptic changes would be expected to change synaptic potency.

An example of an all-or-none synapse that underwent LTP is shown in Fig. 9. Following tetanization, the failure rate decreased significantly from 0.512 to 0.005 ($P < 0.001$, Pearson's χ^2 test), but there was no change of the potency of that synapse. Averaged traces of successive responses before and after the tetanization are essentially identical, showing no difference in amplitude or kinetics. Thus, this synapse provides compelling evidence for a purely presynaptic mechanism of plasticity induced by intracellular tetanization.

At some all-or-none synapses, plasticity induced by intracellular tetanization may be mediated by both presynaptic and postsynaptic mechanisms. This is illustrated in Fig. 10 by an example of an all-or-none synapse that underwent LTD. Following tetanization, the number of response failures increased significantly, and thus the probability of release decreased from 0.951 to 0.728 ($P < 0.001$).

The mean amplitude of successful responses also decreased significantly, from 1.216 ± 0.189 mV ($n = 39$) in control to 0.91 ± 0.242 ($n = 149$) after the tetanization ($P < 0.05$). Thus, LTD at this synapse was due to a combination of both presynaptic and postsynaptic changes.

Summary data for all 24 all-or-none synaptic inputs show that changes of the average EPSP amplitudes,

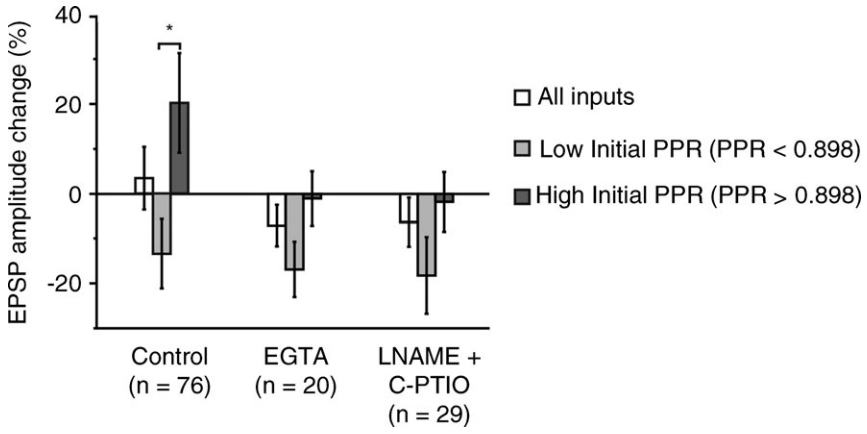

Figure 8. Summary of EPSP amplitude changes under control conditions, EGTA in the intracellular solution, and bath application of L-NAME and carboxy-PTIO
For each condition, averaged changes of all inputs are shown by white bars, averaged changes of inputs with initial PPR < 0.898 are shown by light grey bars, and averaged changes of inputs with initial PPR > 0.898 are shown by dark grey bars. Under control conditions, EPSP amplitude changes at inputs with initial PPR > 0.898 were significantly higher than EPSP amplitude changes at inputs with initial PPR < 0.898 ($P < 0.05$, $n = 38$). Variance of the EPSP amplitude changes was significantly higher in control experiments than in EGTA experiments ($P < 0.05$), and in experiments with L-NAME and carboxy-PTIO application ($P < 0.05$).

calculated including both successful responses and failures, were strongly negatively correlated with the change in the failure rate (Fig. 11*A*, $r = –0.944$, $P < 0.001$). Depressed synapses generally showed an increase in the failure rate, while in potentiated synapses the failure rate decreased. This strong correlation provides clear evidence for involvement of presynaptic mechanisms in plastic changes induced by intracellular tetanization at all-or-none synapses. In contrast, postsynaptic mechanisms contributed little to plastic changes at all-or-none synapses, because average EPSP amplitude changes at these synapses were not significantly correlated with amplitude changes of successful responses (Fig. 11*B*, $r = 0.027$, $P = 0.91$).

Data collected from all-or-none synapses provide further support for the notion that plasticity induced by intracellular tetanization depends on the initial release probability at the presynaptic terminal. Changes of average EPSP amplitudes were correlated with initial failure rates (Fig. 11*C*, $r = 0.519$, $P < 0.01$). Furthermore, initial failure rates were correlated with changes of the failure rate after the tetanization ($r = –0.519$, $P < 0.01$). Thus, the induction of plastic changes at all-or-none synapses was correlated with the initial release probability, and expression of plasticity at these synapses was predominantly presynaptic, with little contribution from postsynaptic mechanisms.

Discussion

Our results demonstrate the following. (1) Long-term plasticity can be induced in layer 2/3 pyramidal cells in rat auditory cortex by a purely postsynaptic challenge, intracellular tetanization. Induction of plasticity by intracellular tetanization required a rise of intracellular [Ca^{2+}]. (2) Direction of the plastic change, potentiation or depression, was correlated with the initial paired-pulse ratio at the synapse. (3) Plastic changes induced by intracellular tetanization involved both pre- and postsynaptic mechanisms. (4) Presynaptic components of plastic changes were abolished in experiments with blockade of NO synthesis and spread, indicating the involvement of NO signalling.

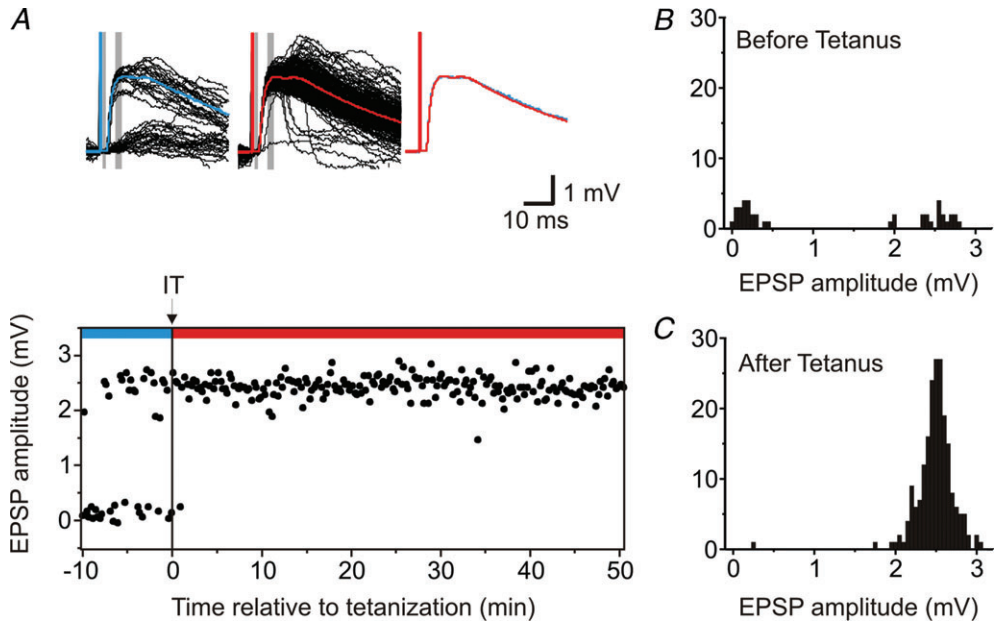

Figure 9. Plasticity of all-or-none responses: example of a potentiation by purely presynaptic mechanisms
A, superposition of individual responses (black traces) during pre-tetanic (blue bar above the response amplitude time course) and post-tetanic (red bar above the time course) intervals. Blue and red traces show averaged responses only (failures excluded). An overlay of averaged responses (on the right) shows that neither the amplitude nor kinetics of responses change after the tetanization. Time course of EPSP amplitude changes. The vertical bar indicates the timing of intracellular tetanization. *B,* histogram of response amplitudes before tetanization. Probability of release $P_r = 0.512$, $n = 41$, mean successful response amplitude (synaptic potency) $A_r = 2.485 \pm 0.057$ mV. *C,* histogram of response amplitudes after tetanization: $P_r = 0.995$, $n = 202$, $A_r = 2.497 \pm 0.014$ mV.

Long-term plasticity in auditory cortex can be induced by a purely postsynaptic challenge

Several lines of evidence indicate that long-term plasticity can be induced by a purely postsynaptic challenge without presynaptic stimulation. Photolytic release of caged calcium inside the cell (Neveu & Zucker, 1996) or intracellular tetanization of the postsynaptic cell had been shown to induce long-term plasticity in the hippocampus (Kuhnt & Voronin, 1994) and in the visual cortex (Volgushev *et al.* 1994, 2000a). Results of the present study add several important components to understanding heterosynaptic plasticity. Induction of plasticity by intracellular tetanization in neurons from auditory cortex shows that this type of plasticity is a general phenomenon in the sensory cortex, not restricted to a specific region. Plasticity induced by intracellular tetanization was triggered by a rise of intracellular calcium concentration, since chelation of intracellular calcium dramatically impaired the induction. Analysis of all-or-none responses provided clear evidence for presynaptic mechanisms involved in plasticity maintenance. Presynaptic changes induced by a purely postsynaptic induction protocol imply retrograde signalling. Indeed, disruption of NO signalling led to a dramatic decrease of plasticity. Finally, disruption of NO signalling also abolished the dependence of the direction of plasticity on initial release probability, indicating that in neurons of the auditory cortex this relationship may rely on presynaptic mechanisms and requires retrograde signalling.

The proportion of inputs which underwent LTP, LTD, or did not change after intracellular tetanization was comparable in the visual cortex (Volgushev *et al.* 2000) and in the present study in the auditory cortex (42%, 33% and 25% in the visual cortex and 28%, 42% and 30% in the auditory cortex). Also the magnitude of the LTP and LTD induced by intracellular tetanization in the auditory cortex neurons ($168 \pm 11.0\%$ and $63 \pm 3.4\%$) was comparable to magnitudes of plastic changes reported for visual cortex neurons ($192 \pm 16\%$ and $52 \pm 4.7\%$). A similar range of EPSP potentiation ($158 \pm 7\%$) was reported in auditory cortex neurons after pairing (Buonomano, 1999). Also LTP and LTD of the field potentials induced in the auditory cortex by tetanic stimulation had similar magnitudes. Tetanic stimulation applied to layer 4 or layer 6 induced a $184 \pm 10\%$ and $151 \pm 8\%$ potentiation of field potentials in layer 2/3 (Kudoh & Shibuki, 1994; Watanabe *et al.* 2007). Low frequency stimulation in layer 6 or local tetanic stimulation in supragranular layers induced a depression of field potential responses to $79 \pm 1\%$ and $75 \pm 1\%$ of the baseline (Kudoh *et al.* 2002; Watanabe *et al.* 2007).

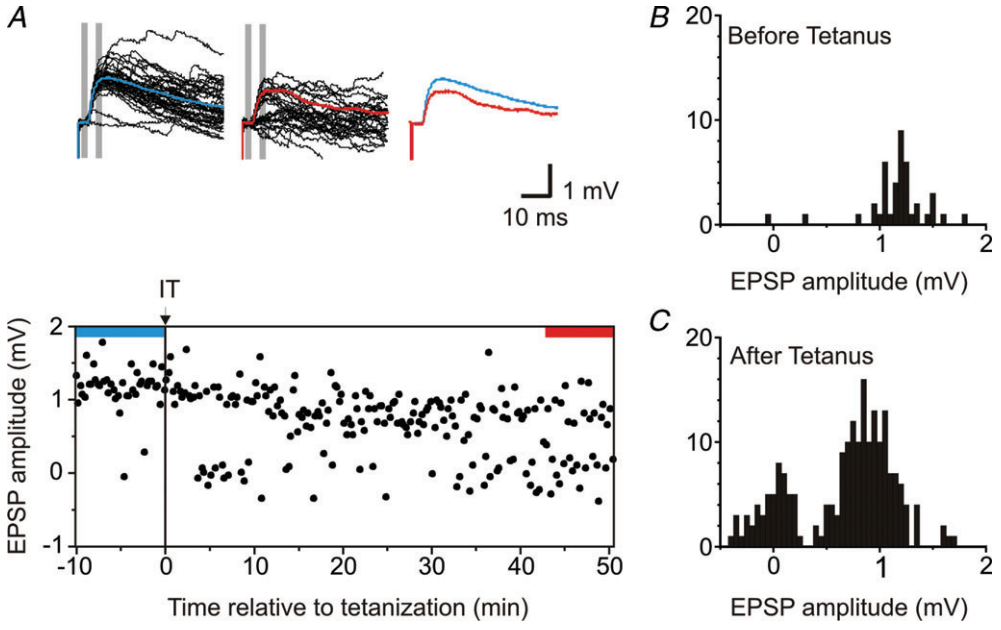

Figure 10. Plasticity of all-or-none responses: example of a depression due to a combination of presynaptic and postsynaptic changes
A, superposition of individual responses (black traces) during pre-tetanic (blue bar above the response amplitude time course) and post-tetanic (red bar above the time course) intervals. Blue and red traces show averaged responses (excluding failures). The overlay of average trace of responses before tetanization (blue trace) and after tetanization (red trace) depicts depression of responses following tetanization. Note that amplitude of successful responses changes. The vertical bar indicates the timing of intracellular tetanization. *B,* histogram of EPSP amplitudes before tetanization. $P_r = 0.951$, $n = 41$, mean successful response amplitude $A_r = 1.216 \pm 0.189$ mV. *C,* histogram of EPSP amplitudes after tetanization. $P_r = 0.775$, $n = 202$, $A_r = 0.911 \pm 0.242$ mV.

Taken together, these results indicate that the ability of purely postsynaptic challenges to induce plasticity is a general property of pyramidal neurons in both visual and auditory cortical areas. Moreover, these results suggest that plasticity may occur not only at the synapses which were active during the induction (homosynaptic plasticity), but also at synapses which were not active during the induction (heterosynaptic plasticity). This form of heterosynaptic plasticity at non-active synapses is different from a 'canonical' form, which accompanies input-specific LTP or LTD, but has an opposite sign and is distance dependent, with its magnitude decaying with increasing distance from the tetanized synapses (Lynch *et al.* 1977; White *et al.* 1990; Royer & Paré, 2003). Induction of plasticity with intracellular tetanization indicates the existence of plasticity mechanisms which may be activated at any distance from the stimulation site, and thus may potentially affect any synapse at a cell. It should be noted that cell firing during intracellular tetanization is well within the range of activity observed *in vivo* during visual stimulation (e.g. Volgushev *et al.* 2003) or pairing of afferent tetanization protocols that are used to induce homosynaptic plasticity (see Fig. 4 and related text in Chistiakova & Volgushev, 2009).

A possible signal which may trigger cell-wide heterosynaptic plasticity is an increase of intracellular calcium concentration caused by backpropagating action potentials. Indeed, once initiated at the axon initial segment, action potentials backpropagate in all dendrites that possess a sufficient density of voltage-gated sodium channels to support active propagation (Stuart & Sakmann, 1994; Stuart *et al.* 1997). Consistent with this hypothesis, our results show that chelation of intracellular calcium by including 10 mM EGTA in the recording pipette led to dramatic impairment of plasticity: both frequency of occurrence and the magnitude of EPSP amplitude changes following intracellular tetanization were reduced. One consequence of the independence of plasticity from the distance to stimulation site is that effects of several strong stimulations sequentially applied at different locations can sum up to reach the threshold for induction of this form of plasticity. Notably, not all synapses underwent plastic changes. A substantial proportion of inputs, 30% in the present study and 25% in the visual cortex (Volgushev *et al.* 2000a) did not change. Although the possibility that some other stimulation protocols may induce plasticity at these synapses cannot be excluded, it is tempting to speculate that these inputs may represent a stable, non-plastic

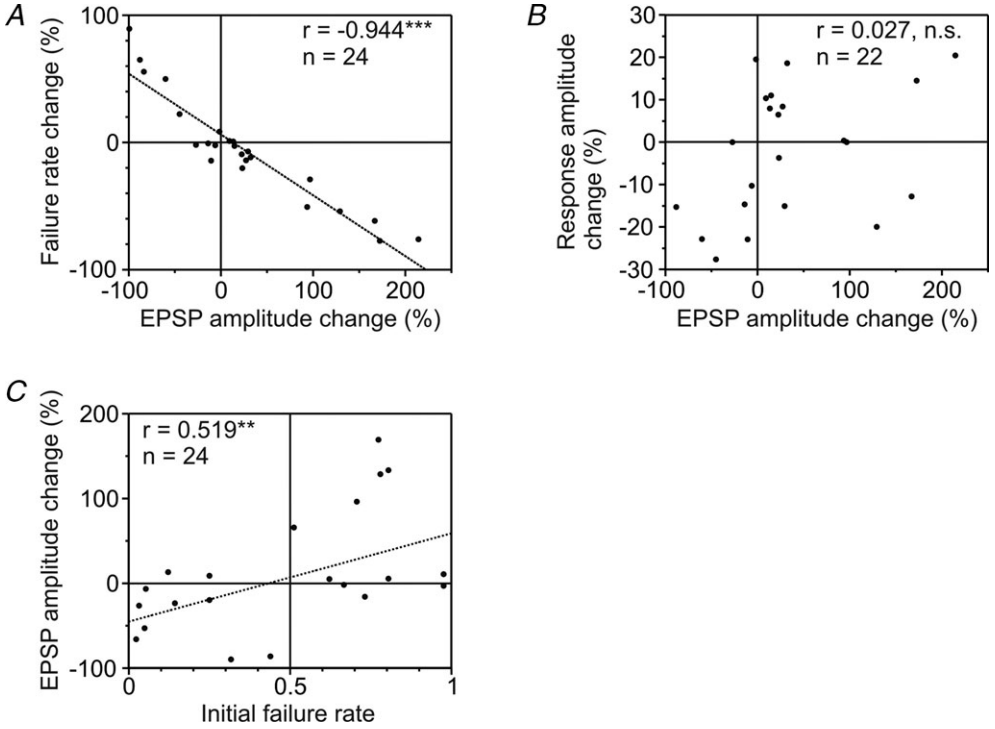

Figure 11. Plastic changes at all-or-none synapses correlate with changes of the failure rate but not synaptic potency
A and *B*, correlation of changes of the average EPSP amplitude calculated including both successive responses and failures, with changes of the failure rate (*A*, $r = -0.944$, $^{***}P < 0.001$, $n = 24$) and changes of the synaptic potency, calculated as averaged amplitude of successful responses (*B*, $r = 0.027$, n.s., $n = 22$). *C*, correlation of tetanization-induced changes of the average EPSP amplitude with failure rate before the tetanization ($r = 0.519$, $^{**}P < 0.01$, $n = 24$).

fraction of synapses required for stable memory storage (Fusi *et al.* 2005).

What determines the direction of synaptic plasticity?

Studies of associative synaptic plasticity have emphasized the role of covariance of activity between the presynaptic and postsynaptic cells (for reviews, see Buonomano & Merzenich, 1998; Malenka & Nicoll, 1999; Holthoff *et al*. 2006; Caporale & Dan, 2008; Feldman, 2009). The covariance hypothesis, an extension of Hebbian rules of associative plasticity, predicts that the direction of plasticity is related to the covariance of presynaptic and postsynaptic activity. In the current study, synaptic plasticity was induced by intracellular tetanization of the postsynaptic cell, without stimulation of afferents during the induction. Some inputs were potentiated, some depressed and some did not change after intracellular tetanization. Since in some cells potentiation of one input but depression of the other occurred simultaneously, the direction of plasticity might depend on the properties of each specific synapse rather than being controlled cell-wide. In neurons of the visual cortex, the direction and magnitude of plasticity following intracellular tetanization were correlated to initial PPR: inputs with initially high PPR having a tendency to be potentiated while inputs with initially low PPR having a tendency to be depressed (Volgushev *et al.* 1997, 2000a). Interestingly, similar dependence of the plasticity direction on the PPR was recently reported for pairing protocols. In a study of plasticity at monosynaptically connected pairs of layer 2/3 pyramids, Hardingham *et al*. (2007) found that pairing induced potentiation at synaptic connections with initially low release probability and high PPR, but depression at synapses with initially high release probability and low PPR. Sáez & Friedlander (2009) replicated this result for monosynaptically connected neurons in layer 4: changes of synaptic strength were positively correlated with initial PPR and initial failure rate. Lu *et al*. (2007) report that excitatory connections between pyramidal neurons and low-threshold spiking interneurons in somatosensory cortex express high PPR and were potentiated after pairing pre- and postsynaptic activation. In contrast, synapses between the same pyramidal neurons and fast-spiking interneurons expressed low PPR and were depressed after the same pairing protocol. Thus, initial release probability at a synapse might be one of the factors determining the direction of plastic changes, or at least one of the indicators of susceptibility of a synapse for potentiation or depression. Consistent with these findings, the direction and magnitude of plasticity induced by intracellular tetanization in auditory cortex neurons was also correlated to initial PPR.

In this study, we have revealed a high proportion of all-or-none synaptic responses (24%). Their properties were very similar to the all-or-none responses described in the visual cortex, but the proportion was higher (about 10% in the visual cortex, Volgushev *et al.* 1995). The direction and magnitude of plasticity at all-or-none synapses was correlated with the initial failure rate. Both PPR and failure rate provide estimates of the probability of vesicle release. Thus, the tendency of intracellular tetanization to induce LTP at synapses with an initially low probability of release, and LTD at synapses with an initially high probability of release was observed for both regular EPSPs and all-or-none synaptic responses.

Plasticity induced by intracellular tetanization depends on the rise of intracellular calcium concentration. The amplitude and kinetics of the rise of intracellular calcium could be one further factor determining direction of plasticity: lower levels of calcium rise leading to depression and higher levels to potentiation (Lisman, 1989; Neveu & Zucker, 1996). Results of experiments with EGTA are consistent with this notion. Indeed, partial chelation of intracellular calcium with EGTA shifted the plasticity balance towards depression.

Because plasticity requires an increase of intracellular calcium, it is possible that inputs that remained stable following intracellular tetanization may represent distal synapses at which local calcium fluxes are reduced due to failure of backpropagating action potentials. However, we did not find a significant relation between EPSP rising slope, which is indicative of the distance of stimulated synapse to the soma, and the direction and magnitude of plastic changes induced by intracellular tetanization. It should be noted that variability of EPSP slopes in our sample was much lower than in recent papers that were aimed at studying the distance dependence of plasticity and used specific stimulation techniques to stimulate synapses at variable dendritic locations (Froemke *et al.* 2005; Gordon *et al.* 2006; Letzkus *et al.* 2006; Sjöström & Häusser, 2006). The similarity of EPSP slopes in our sample indicates that the distances of stimulated synapses from the soma were more homogeneous. Many EPSPs which did not change after intracellular tetanization had similar rising slopes to those expressing plasticity, suggesting that they were located at similar distances from the soma. This makes it unlikely that failure to induce plasticity at about 30% of inputs in this study was due to the failure of backpropagating action potentials to reach only those synapses.

Pre- and postsynaptic mechanisms of synaptic plasticity in auditory cortex

One possible reason why the direction of plastic changes depended on properties of the presynapse is

that mechanisms of plasticity expression were at least partially presynaptic. Indeed, ample evidence indicates the involvement of presynaptic mechanisms in LTP and LTD in somatosensory and visual cortex (e.g. Markram & Tsodyks, 1996; Volgushev *et al.* 2000a; Sjöström *et al.* 2003; Hardingham *et al.* 2007). In the auditory cortex, pairing-induced LTP was associated with changes of paired-pulse ratio, suggesting the involvement of presynaptic mechanisms (Buonomano, 1999). Our results show that presynaptic mechanisms are involved in heterosynaptic LTP and LTD in auditory cortex neurons. EPSP amplitude changes were correlated with changes of CV^{-2} and inversely correlated with changes of the PPR. Since CV^{-2} is proportional to release probability and PPR is inversely proportional to release probability, these correlations indicate that LTP was associated with an increase, and LTD with a decrease, of the release probability.

The separation of involvement of presynaptic and postsynaptic mechanisms in expression of LTP and LTD is most clear in experiments with all-or-none synapses. At these synapses failures can be unambiguously distinguished from successful responses, thus allowing the direct measurement of both the failure rate and the amplitude of successful responses. The direction and magnitude of changes of the averaged EPSP (including both responses and failures) was strongly correlated with changes of the failure rate. In many cases, changes of the average EPSP amplitude were not accompanied by any change of the amplitude or kinetics of successful responses. These cases provide clear evidence for the possibility of purely presynaptic mechanisms of plasticity induced by intracellular tetanization in auditory cortex neurons. In some cases, both the failure rate and the amplitude of successful responses changed. Such cases indicate the involvement of both pre- and postsynaptic mechanisms in plasticity. Presynaptic changes do not exclude the possibility of postsynaptic changes at the same synapse; rather, they may accompany postsynaptic changes.

Because induction of plasticity was postsynaptic, the evidence of presynaptic changes suggests the involvement of a retrograde messenger. Results of experiments involving the blockade of NO production by an inhibitor of NO-synthase, L-NAME, and blockade of NO spread in the extracellular space by a scavenger, carboxy-PTIO, allow us to draw several conclusions. Blocking NO signalling abolished the correlation between changes of EPSP amplitude on the one hand and changes of PPR and and CV^{-2} on the other, suggesting that NO signalling is involved in the biochemical pathway leading to presynaptic changes following intracellular tetanization. Reduction of the magnitude of plastic changes in experiments with disrupted NO signalling indicates that part of the EPSP amplitude changes in control were mediated by presynaptic mechanisms. At the same time, changes of EPSP amplitude after intracellular tetanization that still occurred in experiments with disrupted NO signalling should have been mediated by postsynaptic mechanisms. This latter conclusion is supported by the absence of correlation between the magnitude of EPSP amplitude changes and changes of release indices PPR and CV^{-2}. Finally, blocking NO signalling also abolished the dependence of the direction of plasticity on initial release probability, indicating that in neurons of the auditory cortex this relationship requires retrograde signalling.

A role for heterosynaptic plasticity?

A learning system in which synapses change according to Hebbian-type rules has two major drawbacks: it is prone to runaway dynamics due to a positive feedback intrinsic to the Hebbian learning rule, and it lacks mechanisms of synaptic competition which are necessary for synapse selection (Miller, 1996). These problems can be solved by precisely balancing local learning rules for potentiation and depression (e.g. White *et al.* 1990; Song *et al.* 2000; Song & Abbott, 2001; Sjöström *et al.* 2001; Royer & Paré, 2003). However, a cell-wide mechanism, such as normalization which conserves the total synaptic weight of all synapses at a neuron provides a more robust solution. Normalization of synaptic weights is widely used in theoretical studies since the early models of visual map development (von der Malsburg, 1973). Several reports show that in the visual cortex, the direction and magnitude of synaptic plasticity depend on the initial properties of synapses: synapses with initially low release are more susceptible for potentiation, while synapses with an initially high release probability have a tendency to be depressed or do not change (Volgushev *et al.* 1997, 2000a; Hardingham *et al.* 2007; Sáez & Friedlander, 2009). Here we demonstrate that also in auditory cortex, induction of plasticity by purely postsynaptic challenge depends on the initial properties of synapses. Furthermore, intracellular tetanization induces potentiation and depression at different synapses in a balanced way, so that the net sum of synaptic weights across all synapses remains preserved. This suggests that this cell-wide mechanism for synaptic weight normalization (Chistiakova & Volgushev, 2009) is not an exclusive feature of visual cortex, but extends to auditory cortex.

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Author contributions

The study was designed by M.C. and M.V. Experiments were conducted at the University of Connecticut by C.M.L. and C.S. Data analysis was conducted by C.M.L. and M.V. The paper was written by C.M.L., M.C. and M.V. All authors approved the final version of the manuscript.

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