CHAPTER 3

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Dendritic position is a major determinant of presynaptic strength

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Abstract
Different regulatory principles influence synaptic coupling between neurons, including positional principles. In dendrites of pyramidal neurons, postsynaptic sensitivity depends on synapse location, with distal synapses having the highest gain. Here, we investigated if similar rules exist for presynaptic terminals in mixed networks of pyramidal and dentate gyrus neurons. Unexpectedly, distal synapses had the lowest staining intensities for vesicular proteins vGlut, vGAT, Synaptotagmin and VAMP and for many non-vesicular proteins, including Bassoon, Munc18 and Syntaxin. Concomitantly, distal synapses displayed less vesicle release upon stimulation. This dependence of presynaptic strength on dendritic position persisted after chronically blocking action potential firing and postsynaptic receptors but was markedly reduced on dentate gyrus dendrites compared to pyramidal dendrites. These data reveal a novel rule, independent of neuronal activity, that regulates presynaptic strength according to dendritic position, with the strongest terminals closest to the soma. This gradient is opposite to postsynaptic gradients observed in pyramidal dendrites and different cell types apply this rule to a different extent.
**Introduction**

Presynaptic terminals in the central nervous system, even those formed by a single neuron, form a highly heterogeneous population in terms of both structure and function. Ultrastructural analysis of hippocampal synapses revealed a wide range in the number of synaptic vesicles (40-800 per terminal) and the number of morphologically docked vesicles (0-15 per terminal) [1-3]. A similar variation was observed using functional readouts, including synaptic release probability (pr) [4-7] and the number of release-ready vesicles (the readily releasable vesicle pool, RRP) [2,5]. The pr can change rapidly over time as a result of repetitive stimulation (i.e., short-term plasticity, STP). The direction of change (facilitation or depression) and the extent of STP depend to a large extent on the initial pr and the RRP size, and is therefore synapse-specific as well [6,8]. Indeed, presynaptic terminals from a single neuron express different types of STP [9-11]. In addition, each terminal expresses a unique range of presynaptic receptors. Over 70 presynaptic receptors have been described so far, and most of these receptors modulate pr and STP, via the activation of intracellular signal transduction pathways (see [12]). These forms of synaptic heterogeneity largely increase the computational power of a neuronal network, as every terminal acts as an independent information-processing unit (reviewed in [13,14]).

**Figure 3.1 (opposite page).** Synapse location determines the amount of vesicles per terminal. A. Experimental design. A postsynaptic neuron (post, blue) receives presynaptic input from multiple surrounding presynaptic neurons (pre, grey). The vGlut staining intensity is used as a measure for the number of vesicles per presynaptic terminal (green). Note that a single presynaptic neuron may form multiple synapses along the dendritic tree. B. Typical example of a proximal and distal dendrite stained for vGlut (green) and MAP (blue). Scale bare represents 5 μm. C. Example of an image used for analysis. Scale bare represents 20 μm. D. Analysis of the image in C. The detected neurites are shown in grey. Neurites from neighbouring neurons are omitted from the analysis. The dots represent detected synapses, with their vGlut intensity color coded. The intensity was normalized to the most intense synapse detected in this neuron. The size of the dots does not represent the surface area of the detected synapse. E. Distance mask of the neuron in C, used for distance measurements of the detected synapses. Distance was measured along the MAP staining, and thus takes into account the morphology of the dendritic tree. F-H. Different methods to plot the results of the analysis in B-D. F. Relationship between distance from soma and vGlut intensity. Each dot represents an individual synapse, solid line a linear fit through the data. Inset displays the Spearman correlation coefficient (r) and significance (p). G. Same data as in F, averaged in bins of 20 μm. Error bars represent SEM. H. Cumulative histograms of the data in F, in bins of 20 μm. The color of each line indicates the distance from soma. Legend also applies to panel K and M. P/D is the ratio of proximal (<50 μm) over distal (last 50 μm). I-K. Results from the complete vGlut data set (n=68 cells, 336,620 synapses). I. Relationship between distance from soma and vGlut intensity. Each dot represents an individual synapse, solid line a linear fit through the data. J. Same data as in I, averaged in bins of 20 μm. Error bars are too small to be seen. K. Cumulative histograms of the data in I. P value is the significance of the P/D ratio (one sample Wilcoxon rank test). L. Histogram of the slopes of linear line fits through data of individual cells. M. Cumulative histograms of synapse surface area.
Over the recent years it has become increasingly clear that many of these presynaptic characteristics depend on the dendrite on which the terminal is formed. In hippocampal neurons in vitro, neighboring synapses have similar pr [6,15], which is thought to be specific for a dendritic branch [4]. In slices, pr and STP depend on the identity of the postsynaptic cell [9-11]. Furthermore, the expression of presynaptic receptors depends on synapse location, both in slices [11,16,17] and in culture [18].

The postsynaptic gain, in turn, is largely dependent on the dendritic position of the synapse. In hippocampal pyramidal cells, distal synapses express an increased number of AMPA receptors, leading to larger local postsynaptic currents [19,20]. In addition, synchronous input on distal synapses of hippocampal and cortical pyramidal cells can be amplified by supralinear summation of excitatory postsynaptic synaptic potentials (EPSPs) and the initiation of dendritic calcium spikes [21,22]. Dendritic location is therefore thought to be of crucial importance for the integration of synaptic input (reviewed by [23]). It can be predicted that presynaptic terminals contribute to this amplification by the formation of stronger presynaptic inputs at more distal dendritic positions.

In the current study, we investigated the general relationship between presynaptic strength and the dendritic position of the synapse. Surprisingly, we did not observe the expected increase in presynaptic strength with increasing distance from the postsynaptic soma. In contrast, we found that in both inhibitory and excitatory synapses, the number of synaptic vesicles and the local concentration of presynaptic proteins are highest at synapses formed close to the soma. This clearly affected vesicle release, since proximal synapses had a larger RRP. Strikingly, this distance-dependent scaling of presynaptic strength was insensitive to manipulations of neuronal activity, and was dependent on the identity of the postsynaptic neuron. These results reveal a novel, cell-wide rule that determines the strength of presynaptic input along the dendritic tree. We believe that this will have profound implications for the integration of neuronal activity.

**Results**

*The number of synaptic vesicles depends on synapse location*

We analyzed the amount of vesicles per presynaptic terminal as a function of its location on the dendrite relative to the soma. Cultured hippocampal neurons were stained with antibodies against the vesicular glutamate transporter vGlut (Figure 3.1A-C). We consider vGlut intensity a reliable measure for the relative amount of synaptic vesicles, since the number of vGlut copies per vesicle is tightly controlled [24]. Furthermore, only ~1% vGlut...
is expressed at the cell surface [25]. For every synapse we measured the vGlut intensity and its distance from the postsynaptic soma along the dendritic marker MAP (Figure 3.1C-E). Surprisingly, we found a near perfect gradient of vGlut levels, with proximal synapses having the highest intensity (Figure 3.1F-K). The median normalized intensity dropped from 0.50 at the soma to 0.24 at 250 μm from the soma. Note that considerable variation exists in intensity, even among synapses at similar distance, but that high intensity synapses are more likely to be found close to the soma (Figure 3.1I-K). However, even when binned and shown for individual neurons, a clear relation between distance and intensity of presynaptic staining was observed (Figure 3.1F-H).

To test to what extent the distance dependence of staining intensity holds for every individual neuron, we made linear line fits through the data of each neuron. In 66 of the 68 cells tested, this fit had a negative slope, indicating that all but two cells showed decreased vGlut intensity at distal sites (Figure 3.1L). Figure 3.1L also shows that some heterogeneity probably exists on either side of the median among different cell types in a mixed culture (Figure 3.1L and see below). To further quantify the effect per cell, we calculated the ratio of proximal (<50 μm) over distal (last 50 μm) per cell. This P/D ratio was 1.45 ± 0.04, p < 0.001 (one sample Wilcoxon rank test), again indicating that individual cells display distance-dependent decline in vGlut intensity. The differences in surface area of vGlut puncta between proximal and distal terminals were much smaller, but still significant (median normalized area at the soma: 0.25, at 250 μm: 0.21, Figure 3.1M). It should be noted that, due to the lower vGlut intensity at distal synapses, the area measurements at distal terminals is less precise. These results suggest that the number of vesicles per terminal, and to a lesser extend the physical dimensions of the terminal, depend on the dendritic position of the synapse.

To test if the observed gradients reflect a certain developmental stage rather than a stable state, we compared sister cultures fixed at 14 or 21 days in vitro (DIV). Despite significant differences in average synapse area and synapse density, we did not find any difference in the distance dependency of vGlut intensity of synapse area (Supplemental Figure 3.1). Thus, the distance dependency of vGlut intensity is stable once mature synapses are formed.

Protein content of presynaptic terminals depends on synapse location
Next, we tested the distance dependency of the levels of a range of other presynaptic proteins with immunocytochemistry (Figure 3.2). Synapses were detected with vGlut, synapsin, vGAT or VAMP, and the detected regions were subsequently used to quantify the synaptic levels of other proteins. We observed a distance-dependent decline in the levels of the vesicular GABA transporter vGAT, similar to vGlut (Figure 3.2B). This suggests that the number of vesicles of excitatory and inhibitory synapses is equally dependent on synapse location. Furthermore, similar gradients were found for the presynaptic scaffolding protein Bassoon, and for Munc18, VAMP, Synaptotagmin-1 and Syntaxin, which are core proteins of the vesicle release machinery [26] (Figure 3.2A and B). Similar to our results with vGlut stainings (Figure 3.1), we found only a small drop in surface area at more distal synapses for terminals detected with vGAT, Synapsin or VAMP (Figure 3.2C).
Figure 3.3. RRP size depends on synapse location. A. Experimental design. B. Example traces of synaptic SypHy fluorescence upon stimulation to release the RRP. C. Example image of an Alexa-filled neuron receiving synaptic input from a SypHy+ cell. SypHy image was taken in the presence of 50 mM NH4+. Scale bar represents 20 µm. D. Neurite mask of the neuron in C (grey) with all SypHy+ synapses formed on this neuron. RRP size color-coded. Arrows indicate synapses from which the traces in B were obtained. E. Histogram of RRP size. F. Cumulative histogram of the RRP sizes in E, grouped on synapse location.
Hence, the number of vesicles and the abundance of the release machinery in a terminal, depend on its dendritic location.

**RRP size depends on synapse location**

Next, we tested whether the correlation between protein levels and synapse position translates to functional differences in synaptic release properties. We transfected a fraction of the neurons in the network with SypHy, a fluorescent reporter of synaptic vesicle release [27]. We made electrophysiological recordings of a pair of neurons, of which one was SypHy transfected (which we refer to as the presynaptic cell), and one connected, untransfected cell (referred to as the postsynaptic cell). The postsynaptic cell

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**Figure 3.4. Neuronal activity does not affect distance-dependent scaling of vGlut intensity.**

A. Example images of neurons treated for 7 days with DMSO (control), 10 μM DNQX & 50 μM AP5 (DNQX-AP5) or 2 μM TTX. Scale bar represents 20 μm. B. Average synaptic vGlut intensity. C. Synapse density (number of synapses per μm dendrite). D. Surface area of vGlut puncta. E. Distance dependency of vGlut after DNQX-AP5, TTX or control. Solid lines represent the median normalized intensity, shaded area the 0.4-0.6 boundaries (See also Supplemental Figure 3.1). Control: n=73, 34724 synapses; DNQX-AP5: n=68, 36723 synapses; TTX: n=68, 27417 synapses, N=3. *P<0.05, **P<0.01, compared to control.
cumulative probability
normalized intensity

VAMP
P/D = 1.42±0.04
p < 0.001
N = 5
n (cells) = 119
n (synapses) = 51962

Synapsin
P/D = 1.51±0.06
p < 0.001
N = 4
n (cells) = 64
n (synapses) = 32664

Bassoon
P/D = 1.68±0.13
p < 0.001
N = 2
n (cells) = 35
n (synapses) = 16847

Munc18
P/D = 1.38±0.06
p < 0.001
N = 2
n (cells) = 37
n (synapses) = 20860

Rab3
P/D = 1.88±0.27
p < 0.001
N = 2
n (cells) = 22
n (synapses) = 11410

RIM
P/D = 1.34±0.05
p < 0.001
N = 5
n (cells) = 119
n (synapses) = 51962

Synapsin
P/D = 1.37±0.05
p < 0.001
N = 4
n (cells) = 64
n (synapses) = 32664

distance (µm):
0 140 >260
was filled with Alexa-468 via the recording pipette to observe its morphology (Figure 3.3A and C). To determine the size of the RRP, we stimulated the presynaptic cell with 40 action potentials (AP) at 20 Hz (a stimulation protocol that is routinely used to probe the RRP [3,5,28]), and measured the increase in fluorescence in every terminal in the field of view (Figure 3.2B). The cells where then fixed, and analyzed at a confocal microscope to identify the terminals formed on the postsynaptic cell (Figure 3.3D). The RRP size of these synapses followed a skewed distribution (Figure 3.3E), in good agreement with previous studies [5] [6].

In line with our data on presynaptic protein content, we found that proximal synapses had a larger RRP than distal synapses (Figure 3.3F). The median normalized RRP size ranged from 0.39 for synapses at the soma to 0.19 for synapses formed at >160 \( \mu \text{m} \) from the soma. The P/D ratio per cell was 1.55 ± 0.19 (p < 0.01). Furthermore, application of 50 mM NH4+ (which de-quenches SypHy in unreleased vesicles [29]) confirmed the relationship between number of vesicles and synapse position that we observed in fixed neurons (S/D ratio = 1.15 ± 0.18, p < 0.01, Supplemental Figure 3.2). These results indicate that the increased vesicle number and release machinery abundance translates into larger releasable vesicle pools in nerve terminals at proximal dendritic positions.

**Distance dependency of presynaptic strength is independent of network activity**

Previous studies have shown that neuronal activity fine-tunes synaptic strength [2,5,30,31] and, for synapses onto single dendritic branches, reduces the heterogeneity in pr [4]. Furthermore, manipulations of network activity alter synaptic protein levels [5,32]. The distance-dependent scaling of presynaptic strength could be caused by local differences in dendritic activity, which causes homeostatic adaptations of pr [4]. We therefore tested the effect of blocking AP firing with TTX, or blockage of excitatory synaptic transmission with DNQX & AP5 on distance-dependent scaling of presynaptic terminals. TTX or DNQX & AP5 were added at DIV 7, and samples were fixed at DIV 14 (Figure 3.4A). Both treatments had a substantial effect on average synapse size and vesicle content (Figure 3.4B-D), as observed before [2,4,32], but the correlation between vGlut intensity and synapse position remained (Figure 3.4E). In line with this observation, we did not find any significant differences in the P/D ratio per cell (p = 0.81, Figure 3.4F), or in the slope of the linear line fit per cell (p = 0.94, Figure 3.4G).

To further test the effect of network activity, we measured the distance dependency of presynaptic protein levels in autaptic islands. These neurons are grown in complete

Figure 3.5 (previous page). Autaptic islands display distance-dependent decline in presynaptic protein levels. Scale bars represent 20 \( \mu \text{m} \). A. Example images of hippocampal autaptic cultures stained for synapse marker Synapsin or VAMP and additional synaptic proteins. GFP expression (delivered with lenti virus) was used as dendritic marker. B. Cumulative histograms of the synaptic intensity of proteins involved in vesicle release. Synapses were grouped based on their distance from the postsynaptic soma. All intensities were normalized to the largest value per cell. P/D is the ratio of proximal (<50 \( \mu \text{m} \)) over distal (last 50 \( \mu \text{m} \)), p value is the significance of the P/D ratio. N represents the number of independent experiments. C. Surface area of synapsin and VAMP puncta. Abbreviations: BSN: Bassoon; M18: Munc18.
Figure 3.6. Strongly reduced distance-dependent scaling in DG cells. A. Example images of proximal and distal synapses in DG and non DG cells. Scale bars represent 5 μm. B. Cumulative histograms of normalized vGlut intensity of non DG cells. vGlut intensity was normalized to the largest synapse per cell. P/D is the ratio of proximal (<50 μm) over distal (last 50 μm), p value is the significance of the P/D ratio. N represents the number of independent experiments. C. As B, for DG cells. D. Comparison of the histograms in B and C. Solid line represents the median, shaded area the 0.4 – 0.6 boundaries. E. P/D ratio of vGlut intensity of DG and non-DG cells. F. Average slopes of linear line fits through data from individual neurons. ** p < 0.01.
isolation, and thus are devoid of network activity [33]. Furthermore, since all presynaptic terminals on an island are formed by the same axon, every synapse is expected to receive a similar activity pattern. This type of cultures is routinely used to study presynaptic function (see for instance [6,34,35]). In these autaptic cultures, we found distance-dependent gradients for all presynaptic proteins tested, both vesicular and non-vesicular, in a similar fashion as observed in network cultures (Figure 3.5). Thus, the dependence of vesicle number and secretion machinery abundance on dendritic position is independent of synaptic activity or differences in local postsynaptic current amplitude.

Protein levels are not correlated in individual synapse
If the local concentration of release machinery proteins is indicative for presynaptic strength, it could be predicted that their levels are correlated at the level of individual synapses. We therefore tested whether such a correlation exists within the co-stainings described above. As can be expected, the levels of vesicular proteins showed a good correlation: Spearman correlation coefficient (r) of VAMP vs. Synaptotagmin = 0.59; VAMP vs. Rab3 r = 0.74. Furthermore, Bassoon intensities correlated well with Synapsin (r = 0.63) and vGlut (r = 0.61), while RIM and Synapsin showed weaker correlation (r = 0.46). However, VAMP levels did almost no correlation with either Munc18 (r = 0.29) or Syntaxin (r = 0.22). These results show that although these proteins display a distance-dependent effect on the population level, differences between individual proteins of the release machinery exist in individual synapses.

Distance-dependent scaling is strongly reduced in dentate gyrus granule cells
Our data suggest that the previously observed increased postsynaptic gain at distal synapses [19,22] is counterbalanced by weaker presynaptic inputs. Interestingly, it was shown that dendrites of granule cells in the dentate gyrus (DG cells) integrate synaptic input independent of synapse location [36]. We hypothesized that if distance-dependent scaling of presynaptic strength is important for the integration of synaptic input, pyramidal and dentate gyrus cells might also scale their synaptic input differently. Previous work has shown that hippocampal neurons retain their morphological and functional characteristics in culture [37-39]. DG cells can be identified in culture using antibodies against the transcription factor Prox1; the remaining cell population (non-DG cells) consists of pyramidal cells from the cornu ammonis and a small fraction of interneurons [39,40]. We confirmed the specificity of Prox1 by morphological analysis of DG and non-DG cells. Comparable to in vivo [41-43], DG cells in culture had a smaller soma and a smaller and less complex dendritic tree (Supplemental Figure 3.3A-H). We then quantified the vGlut intensity to measure distance-dependent scaling in both cell populations (Figure 3.6). In non-DG cells, we found a strong reduction of vGlut intensity in more distal synapses (normalized median intensity at the soma: 0.39, at 260 μm: 0.26), consistent with our previous experiments (Figure 3.6B). Strikingly, distance-dependent scaling was strongly reduced in DG cells (normalized intensity at the soma: 0.39, at 260 μm: 0.31; Figure 3.6C-D). Even though proximal synapses were still slightly more intense than distal synapses (P/D ratio = 1.15 ± 0.06, p <0.001), this was significantly smaller compared to non-DG cells (p < 0.01, Figure 3.6E). To further test this, we made line fits through the data of individual neurons. The slope of this fits was significantly smaller in DG cells (p < 0.01, Figure 3.6F), again indicating that distance-dependent scaling is reduced in these cells.
cells. The difference between both groups was even more pronounced when comparing absolute vGlut intensities (median intensity soma vs. distal: 706.6 vs. 409.1 AU (non-DG); 518.1 vs. 690.8 (DG), Supplemental Figure 3.3I-J). These results suggest that populations of neurons that display different dendritic integration principles also have different rules for distance-dependent scaling of presynaptic input.

**Discussion**

It is well established that the number of vesicles, release machinery abundance and pr can vary widely between presynaptic terminals [3,6,32]. Since the regulation of presynaptic efficacy is a crucial element in neuronal information processing and memory formation [14], the strength of presynaptic terminals is expected to be dictated by tight rules. In cortical and hippocampal pyramidal neurons, the local amplitude and integration rules of postsynaptic currents are controlled by synapse location on the dendrite, with distal synapses having the highest gain [19,22]. We predicted a similar rule for the strength of presynaptic input, to further amplify the signal of distal synapses. Surprisingly, we found the exact opposite.

**Presynaptic strength depends on the location of the synapse**

The synaptic levels of the vesicular proteins vGlut, vGAT, Synaptotagmin and VAMP decreased with increasing distance from the postsynaptic soma (Figure 3.1 & 2), from which we conclude that distal synapses have less vesicles. If vesicles at distal synapses would contain fewer copies of these proteins, that might also contribute to this distance dependent differences in staining intensity. However, this is unlikely, since synaptic vesicles actively and rapidly mix between different presynaptic terminals via axonal transport [1,44,45] and single vesicle quantification of vesicle content showed that the number of vGlut and Synaptotagmin molecules per vesicle shows little variation [24]. In addition to differences in the number of vesicles per terminal, we found similar distance-dependent differences in the local concentration of non-vesicular proteins essential for synaptic vesicle release (Figure 3.2 and 4).

At the level of individual synapses, we observed moderate correlations in the levels of various vesicular proteins, and of scaffold proteins and vesicular markers. However, the levels of other release-machinery proteins did not correlate well. Thus, although the concentration of these proteins show a distance-dependent effect on the population level, large differences between individual proteins do occur in single synapses. In most experiments described here, presynaptic terminals on a given dendrite originate from several neighboring neurons. As each presynaptic neuron has its independent gene expression program, the relationship between the expression of different presynaptic proteins is expected to be different in different subsets of presynaptic terminals. Furthermore, recent studies showed that in response to neuronal activity, synaptic levels of specific proteins are regulated, while other proteins are unaffected [32,46]. For these reasons, the levels of various presynaptic proteins are not expected to show strong correlations.

The distance-dependent regulation of the number of vesicles and release-machinery proteins probably account for the distance dependency of the RRP size (Figure 3.3).
Indeed, alterations in Munc18 and Bassoon levels are known to correlate with changes in RRP size [5,35]. Since the size of the RRP is a primary determinant of pr and STP [6,47], these differences in RRP size will have a profound effect on vesicle release during neuronal activity.

The data described above seem in conflict with previous studies, which did not find a relationship between pr or RRP size and distance from the postsynaptic soma [4,20]. Our results display a broad heterogeneity in RRP size, vesicle number and protein levels, even between synapses with a similar distance from the soma (Figure 3.1-3). This variation will make a distance-dependent effect on presynaptic strength hard to detect using low throughput methods. In our SypHy experiments we were able to measure the RRP size of 1513 synapses from 17 cells. With this relatively large number of synapses per cell we observed a clear distance-dependent decline in RRP size (Figure 3.3). The apparent discrepancy between previous studies and our results might thus be due to an increased power of the methods used here. Our results also clearly illustrate that, besides synapse position, other factors are involved in the regulation of presynaptic strength. The identity and activity of the presynaptic cell, as well the activation of intracellular signal transduction pathways are factors known to be involved in the regulation of presynaptic vesicle release [48,49]. In a recent study, it was found that terminals formed by the distal part of the axon were stronger compared to proximal synapses, suggesting presynaptic strength might also depend on the axonal position of a terminal [50]. Taken together, the observed pr of a given synapse is the result of all the determinants mentioned above, and cannot be solely attributed to a single factor.

Figure 3.7. Pre- and postsynaptic strength are determined by several independent mechanisms. Chronic changes in synaptic activity lead to compensatory adaptations in synaptic strength (homeostatic plasticity). These mechanisms act locally, at the level of individual synapses. On top of this, the strength of the pre- and postsynapse is scaled in a cell-wide manner, based on the synapse’s position on the dendritic tree (distance-dependent scaling). Both mechanisms act in parallel, most likely via independent molecular mechanisms. The distance-dependent scaling is cell-type specific, and may act in conjunction with the integration rules of the cell’s dendritic tree.
Distance-dependent scaling is dictated by the postsynaptic cell
The synapses studied here are formed by axons from several neighboring neurons. Thus, each axon is capable of determining the location of a synapse on the dendritic tree, and to set its presynaptic strength accordingly. It is therefore most likely that the postsynaptic cell dictates the distance-dependent scaling of presynaptic strength, by indicating the position of the synapse on the dendrite via retrograde messenger or cell adhesion molecules. In contrast to known forms of homeostatic plasticity [30,31], distance-dependent scaling is independent of neuronal activity (Figures 4 & 5). Therefore, this retrograde signaling to tune presynaptic strength is most likely distinct from the previously documented, activity dependent signaling pathways (reviewed by [51]). We postulate that in parallel to local homeostatic mechanisms, presynaptic strength is determined by cell-wide, distance-dependent scaling, which is controlled by the postsynaptic cell (Figure 3.7). Distance-dependent scaling might act as a global, default rule, which is stable over time and insensitive to activity in the neuronal network. Homeostatic mechanisms, in turn, respond to changes in neuronal activity, and tune individual synapses based on their synaptic use. Together, distance-dependent and homeostatic mechanisms ensure the proper integration of synaptic input with optimal use of metabolic resources [13].

Distance-dependent scaling is cell-type specific
Our results indicate that the gradient of presynaptic strength is the opposite of the previously observed distance-dependent increase in postsynaptic gain in hippocampal pyramidal cells [19,22]. The location-specific integration rules for synaptic input are thought to be of high importance for information processing and memory storage in these cells [23]. Many of these rules, however, are specific for particular cell types (compare for instance [52] and [19]). Importantly, DG cells were shown to integrate synaptic input differently compared to hippocampal pyramidal cells [36]. The absence of dendritic spikes and the strong attenuation of both EPSPs and back-propagating APs result in a linear summation of synaptic input in DG granule neurons, with similar gain for proximal and distal synapses. It was estimated that DG cells require input from ~55 synapses to reach their AP threshold, compared to only 5 synchronous EPSPs in hippocampal pyramidal cells [36]. A reduction of presynaptic strength as a function of distance would further reduce the AP firing probability caused by physiological presynaptic activity. By comparing DG and non-DG neurons we found that, comparable to postsynaptic gain, the distance-dependent scaling of presynaptic strength was significantly reduced in DG cells (Figure 3.6 and Supplemental Figure 3.3). The location-specific scaling of presynaptic terminals thus was different between different populations of neurons. This illustrates that distance-dependent scaling of presynaptic strength is a cell-type specific property, and neurons appear to tune the strength of their afferent input in conjunction with the cell type-specific integration rules of their dendritic tree.

Distance-dependent scaling is expected to regulate complex network performance
Our data reveal a new rule that regulates the strength of presynaptic terminals formed along the dendritic tree of hippocampal neurons. Since synaptic integration rules are highly dependent on dendritic location [23], this may be of crucial importance for the proper integration of synaptic activity. In the intact hippocampus, different regions of
the dendritic tree receive inputs from different anatomical sources (reviewed in reference 55). Combined with our data, it can be predicted that these inputs differ in presynaptic strength (and probably also STP), based on their dendritic location. In line with this prediction, [53] showed that the perforant path (which projects on distal dendrites of CA1 pyramidal cells) display a low pr and paired-pulse facilitation, while Schaffer collaterals, projecting on proximal dendrites, have a higher pr and show frequency depression (but also see [54]). Together with the postsynaptic scaling described before, these distance-dependent rules imply that the power of these anatomical sources to influence the activity of a target neuron depend on the position of their terminals on the dendrite they project on. To what extend distance-dependent scaling affects the strength of other inputs, and how this influences information processing in the hippocampus, remains an important open question.
References


**Supplemental Figure 3.1 (opposite page).** Distance-dependent scaling of total pool size is independent of neuronal development. A. To compare distance-binned histograms of different experimental groups, we made profiles of each histogram. For each distance bin we took the fluorescence intensity that corresponds with a cumulative probability of 0.4, 0.5 (the median) and 0.6, which is shown in B. B. Profile of the distance-binned histogram in A. Solid line is the median, shaded area represents the 0.4-0.6 boundaries. C. Average synaptic vGlut intensity per cell for neurons fixed at DIV14 or DIV21. D. Average synapse area. E. Average synapse density per cell. F. Average dendrite length per cell. G. Cumulative histograms of vGlut intensity of cells fixed at DIV14, grouped by distance from postsynaptic soma. Intensity was normalized to the largest synapse formed on the cell. Legend applies to all panels. H. As G, for neurons fixed at DIV21. I. Comparison of histograms in G and H. J. Cumulative histograms of area of synapses in E. K. Cumulative histograms of area of synapses from H. L. Comparison of histograms from J and K. * p < 0.05, ** p < 0.01.
Supplemental Figure 3.2. Total vesicle pool size is related to RRP size and depends on synapse position. A. Representative traces of SypHy fluorescence from single synapses stimulated with 40AP at 20Hz to release the RRP. B. Traces from the same synapses as in A, stimulated with 900AP at 20Hz to release the releasable pool, and NH4+ to visualize the pool of unreleased vesicles. C. Histogram of the RRP size as measured from the SypHy fluorescence. D. Total pool size from the same synapses in C, measured from the response to 900AP + NH4+. E. Typical example of the relationship between RRP size and total pool size for synapses formed by one neuron. F. Relationship between distance from postsynaptic soma and the size of the total vesicle pool. Data from 12 cells (1066 synapses), N = 3.
Supplemental Figure 3.3. Dentate gyrus granule cells retain their identity in culture and show strongly reduced distance-dependent scaling. A. Example images of a dentate gyrus (DG) cell and non DG cell in culture. Scale bars represent 20 µm. B-H. Morphological characterization of DG and non DG cells. B. Sholl analysis of dendritic branching. Legend applies to all panels. C. Surface area of the soma. D. Total dendrite length in the field of view. E. Number of synapses in the field of view. F. Synapse density (number of synapses per µm dendrite). G. Surface area of synapses. H. Average vGlut intensity of all synapses. Data was obtained from 73 non DG and 77 DG cells, N = 4. All data is expressed as average, error bars represent SEM. * p < 0.05, *** p < 0.001. I. Cumulative histograms of absolute vGlut intensity of DG (right) and non DG cells (left). Synapses were grouped by distance from postsynaptic soma in bin of 20 µm. J. Comparison of the histograms in I.