Determinants of synaptic strength vary across an axon arbor

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Peng X, Parsons TD, Balice-Gordon RJ. Determinants of synaptic strength vary across an axon arbor. J Neurophysiol 107: 2430–2441, 2012. First published January 25, 2012; doi:10.1152/jn.00615.2011.—We used synaptophysin-pHluorin expressed in hippocampal neurons to address how functional properties of terminals, namely, evoked release, total vesicle pool size, and release fraction, vary spatially across individual axon arbors. Consistent with previous reports, over short arbor distances (~100 µm), evoked release was spatially heterogeneous when terminals contacted different postsynaptic dendrites or neurons. Regardless of the postsynaptic configuration, the evoked release and total vesicle pool size were highly correlated with the amount of NMDA receptors and PSD-95 in postsynaptic specialization. However, when individual axons were followed over longer distances (several hundred micrometers), a significant increase in evoked release was observed distally that was associated with an increased release fraction in distal terminals. The increase in distal release fraction can be accounted for by changes in individual vesicle release probability as well as readily releasable pool size. Our results suggest that for a single axon arbor, presynaptic strength indicated by evoked release over short distances is correlated with heterogeneity in total vesicle pool size, whereas over longer distances presynaptic strength is correlated with the spatial modulation of release fraction. Thus the mechanisms that determine synaptic strength differ depending on spatial scale.

synaptic vesicle; release properties; vesicle pool; hippocampus; presynaptic

THE ABILITY OF HIPPOCAMPAL NEURONS to differentially regulate presynaptic strength at terminals located along the same axonal branch is thought to underlie reliable neurotransmission in the presence of highly variable stimuli. Several studies have documented the heterogeneous functional properties among presynaptic terminals of initial release probability (Hessler et al. 1993; Huang and Stevens 1997; Murthy et al. 1997; Rosenmund et al. 1993). Recent studies suggest that postsynaptic neurons (Koester and Johnston 2005; Murthy et al. 1997; Rosenmund et al. 1993) can specify different presynaptic functional properties. While central nervous system (CNS) axons are usually up to tens of micrometers long and making synapses on many targets, how functional properties are distributed along individual long axon arbors and how these are associated with postsynaptic specializations remain unclear.

Here we address two specific questions: what is the spatial distribution of presynaptic functional properties along an axon arbor, specifically the evoked release, total vesicle pool size, and release fraction, as assayed by changes in synaptophysin-pHluorin fluorescence? What is the relationship among presynaptic functional properties and the composition of postsynaptic specializations? The work we report shows that the answers to these questions are different depending on spatial scale. Over short axon segments, evoked release is correlated with total vesicle pool size and is related to the amount of postsynaptic components such as NMDA receptors and PSD-95. Over the entire axon arbor, evoked release is correlated with proximal-to-distal changes in release fraction, independent of total vesicle pool size, that can be correlated for changes in individual vesicle release probability and/or readily releasable pool size. Our results thus suggest that the mechanisms that establish synaptic strength vary over different spatial scales.

MATERIALS AND METHODS

Animals. All of the studies reported were submitted to, and approved by, the University of Pennsylvania’s Institutional Animal Care and Use Committee.

Cell culture. Primary neurons cocultured with astrocytes were prepared as described previously (Elmariah et al. 2005), with minor modifications. Briefly, hippocampi were dissected from embryonic day 18 rats, dissociated in Hanks’ balanced salt solution (with MgCl2 and HEPES; Invitrogen, Grand Island, NY) containing 1% papain for 20 min, triturated in basal medium Eagle (Invitrogen), and plated at 75,000 cells/ml on poly-L-lysine (1 mg/ml; Sigma, St. Louis, MO)-coated coverslips with grids (Bellco Biotechnology) in 24-well plates. Cells were grown at 37°C, 5% CO2, 95% humidity in Neurobasal medium (Invitrogen) plus B27 (Invitrogen) and fetal bovine serum (Hyclone; to sustain astrocytes in the first week) that was changed weekly. Neurons cocultured with astrocytes instead of those treated with astrocyte conditioned medium were used, because contact with astrocytes has been shown to increase the number of functional presynaptic terminals in vitro (Elmariah et al. 2004; Hama et al. 2004).

Constructs and transfection. The synaptophysin-pHluorin construct, in which one copy of the pHluorin sequence was inserted into
the luminal loop of the mouse synaptophysin sequence, was obtained from Dr. Y. Zhu (Zhu et al. 2009). Primary hippocampus neuron cultures were transfected with this construct (0.03%/0.06 μg DNA) using Lipofectamine 2000 (0.06%/0.12 μg per coverslip; Invitrogen) at 7 days in vitro (DIV) at very low efficiency (<5–10 synaptophysin-pHluorin+ neurons per coverslip) and were imaged 7–10 days after transfection. In some experiments, neurons were cotransfected with mCherry (obtained from Dr. R. Y. Tsien) to identify axons from individual neurons. Synaptophysin-pHluorin was localized primarily to axons, accumulated at presynaptic terminals. Immunostaining showed that total pHluorin fluorescence was linearly correlated with immunostaining fluorescence of the endogenous synaptic vesicle markers SV2 and vGlut at each terminal (data not shown).

Live imaging and extracellular stimulation. At 14–17 DIV, coverslips were mounted in a customized imaging chamber (modified RC26 chamber; Warner Instruments). Coverslips were constantly perfused with physiological saline solution containing (in mM) 119 NaCl, 2.5 KCl, 2 CaCl2, 1.3 MgCl2, 25 HEPES (pH 7.4), and 30 glucose. NH4Cl saline solution (pH 7.4) was prepared by substituting 50 mM NaCl in normal saline with NH4Cl. In experiments to measure evoked release during 1-Hz 70-s stimulation, bafilomycin A1 was included in the bath to block the reacidification of recycled vesicles (Calbiochem; 0.25 μM) so that measurement of evoked release would be accurate during 1-Hz stimulation. Bafilomycin was not included in other stimulation frequencies to ensure repeated measurement of the same set of presynaptic terminals. Measurement of evoked release in 20-Hz 3.5-s stimulation was less biased by reacidification without bafilomycin (Atluri and Ryan 2006; Zhu et al. 2009). An in-line heater (Warner Instruments) was used to maintain the solution in the chamber at ~35°C. The chamber was mounted onto an inverted microscope (Leica DMi3000B), and neurons were imaged with a ×63, 1.2-numerical aperture objective using a Hamamatsu cooled charge-coupled device camera (C9100) and appropriate fluorescence excitation and emission filters (N2.1 filter cube for mCherry, GFP filter cube for pHluorin; Leica), and the incident light was attenuated so that photobleaching was minimal or absent during the imaging session. Only glutamatergic neurons that had complex dendritic arbors, short dendritic segment lengths, and relatively more round cell bodies under differential interference contrast illumination (c.f. Benson et al. 1994) were imaged. In some experiments, the absence of glutamic acid decarboxylase staining confirmed that cells with this morphology were glutamatergic (data not shown). Axon segments with synaptophysin-pHluorin-labeled presynaptic terminals were selected for imaging on the basis of their presumptive apposition to postsynaptic dendrites, confirmed via post hoc immunostaining for the dendritic marker MAP2 (Fig. 1A). Axon segments were stimulated with a glass pipette (0.5–0.9 MΩ) placed near an axon of interest at 20 Hz using 1-ms, 400–800-μA square pulses in trains of 3.5-s duration if not stated otherwise. These stimulation parameters were chosen to deplete the readily releasable pool of synaptic vesicles (Burrone et al. 2006; Pyle et al. 2000; Schikorski and Stevens 2001; Waters and Smith 2002) without a significant fluorescence decrease associated with endocytosis and reacidification (Atluri and Ryan 2006; Zhu et al. 2009). Pulses were delivered through Iso-Flex isolator controlled by a Master-8 (AMPI).

Stimulation parameters were chosen to maximize the presynaptic response and also to saturate the image field. Over a range of stimulation amplitudes from 50 to 1,200 μA, we found that presynaptic responses began to saturate at about 600 μA (data not shown). When the stimulation exceeded 1,000 μA, damage to axons was noted (i.e., fluorescence did not return to baseline after 5 min.). Thus 400–800 μA was used for experiments. To determine whether a presynaptic terminal’s response varied significantly with electrode location, the electrode was placed at two positions within a field and terminal responses were compared in two trials at least 5 min apart. The ratio of evoked release between trials 1 and 2 was ~1 with a correlation coefficient $r = 0.9$. These data suggest that presynaptic responses are replicable and are independent of electrode placement.

When multiple segments of axon arbors were measured, segments were at least 300 μm away from each other. The presynaptic terminals clusters of vesicles were visible at rest without stimulation due to baseline fluorescence. Electrical stimulation was applied segment by segment until presynaptic terminals responsive to the stimulation were located. The most proximal axons segments were those closest to the cell body that show evoked release in response to 20-Hz stimulation. These segments were usually a few hundred micrometers away from the cell body (on average, 650 ± 337 μm from cell bodies). Axon segments that were closer to the cell body but did not contain presynaptic terminals that responded to stimulation were not included. Axon arbors were sampled until reaching the growth cone or a point where ambiguously tracking an axon was no longer possible. The most distal segments that were possible to track are defined as distal segments, and these were typically 500 to >1,000 μm from the most proximal axon segment that was assessed (on average, 2,163 ± 593 μm from cell bodies). Stimulation progressed either proximal to distal or vice versa in different neurons.

Coverslips were perfused with NH4Cl saline to deacidify synaptic vesicles, allowing measurement of the maximum fluorescence change (ΔF) that reflects the total pool of vesicles clustered at each presynaptic terminal (Burrone et al. 2006; Sankaranarayanan et al. 2000). This was followed by acidic saline to quench surface fluorescence. The surface fluorescence fraction was measured as surface fluorescence divided by total fluorescence (Wienisch and Kinga 2006). The pHluorin surface fraction was, on average, 13.7 ± 1.0% (51 terminals, 4 neurons) and was stable over the time course of experiments. Thus ΔF after NH4Cl perfusion in our experiment mainly reflects the total vesicle pool size among presynaptic terminals.

Time-lapse images were acquired using Metamorph (Molecular Devices) controlling a Lambda SC Smart shutter (Sutter Instrument) at 2 Hz for evoked release and at 0.5 Hz for NH4Cl saline perfusion to visualize all synaptophysin-pHluorin+ clusters and total vesicle pool size. At the end of the experiment, a bright-field image was taken to document the field of interest, and coverslips were fixed and processed for subsequent immunostaining.

Immunostaining. Coverslips were fixed in 4% paraformaldehyde and 4% sucrose at room temperature for 15 min and rinsed in PBS with 0.25% Triton X-100. For anti-NR1 immunostaining, coverslips were fixed in 4% paraformaldehyde and 4% sucrose at room temperature for 1 min, followed by MeOH at −20°C for 4 min. Coverslips were rinsed in PBS and blocked in PBS containing 5% normal goat serum. Coverslips were then incubated in one or more of the following primary antibodies: anti-PSD-95 (mouse monoclonal; Affinity BioReagents, Golden, CO), anti-MAP2 (mouse polyclonal; gift from Dr. V. Lee), anti-NR1 (rabbit polyclonal; Sigma), anti-GluR1 (rabbit polyclonal; Chemicon, MO), anti-vGlut (guinea pig polyclonal, Chemicon, MO), anti-SV2 (mouse monoclonal; Developmental Studies Hybridoma Bank, Iowa City, IA). Antibodies were visualized after staining with the appropriate FITC-, TRITC-, or Cy5-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA). After immunostaining, the region of interest (ROI) was located and imaged using a confocal microscope (Leica TCS 4D). Field size and pixel resolution were adjusted so that confocal images were in register with live images pixel by pixel. In each image, laser light levels and detector gain and offset were adjusted so that no pixel values were saturated or values were readable at the range of the image. Using ImageJ software (adapted from Bergsman et al. 2006). The major advantage of this algorithm is that a wide range of synaptic

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Evoked release of presynaptic terminals is heterogeneous in short axon segments. A, top: representative images of synaptophysin-pHluorin\(^+\) synaptic vesicle clusters (green) along an local axon segment from a 14-day in vitro (DIV) neuron after live imaging followed by fixation and immunostaining for MAP2 (red). All of the terminals analyzed in this segment were apposed to neuronal dendrites from multiple postsynaptic neurons. Arrows and arrowheads indicate all presynaptic terminals in this axon segment (defined using criteria described in MATERIALS AND METHODS): arrows indicate releasing terminals, and arrowheads indicate nonreleasing terminals. Terminals 1–4 indicate the releasing or nonreleasing presynaptic terminals shown in the pseudocolored time-lapse image series (bottom). The cluster labeled with an asterisk was trafficked to that location in the interval between when the NH4Cl image was taken and fixation and was excluded from the analysis. Scale bar, 10 \(\mu m\). Bottom: pseudocolored time-lapse images of presynaptic terminals 1–4 taken before, during (thick horizontal bar, 3.5-s duration), and after electrical stimulation. The changes in fluorescence (\(\Delta F\)) of evoked vesicle exocytosis (evoked release) at terminals 1–4 are measured as fluorescence by the end of stimulation minus baseline fluorescence (also refer to METHODS AND MATERIALS). The color scale is in arbitrary fluorescence units (AFU). B: total fluorescence of individual presynaptic terminals plotted against time to show \(\Delta F\) during stimulation. The stimulation duration is indicated by the thick horizontal bar below the \(x\)-axis from 3.5 to 7 s. The \(\Delta F\) values of presynaptic terminals 1–4 are heterogeneous, varying over a >10-fold range; e.g., \(\Delta F\) for terminal 2 was 30.9 \(\times 10^4\) AFU, whereas \(\Delta F\) for terminal 3 was 3.4 \(\times 10^4\) AFU. Fluorescence returned to baseline within 1–2 min after stimulation ceased, as synaptophysin-pHfluorin\(^+\) vesicles were endocytosed and reacidified (\(r = 16.1 \pm 0.3\) s, 11 presynaptic terminals; comparable to Sankaranarayanan and Ryan 2000). C–E: comparison of pair ratios. C: the pair ratios of evoked release for terminals innervating the same dendrite were not significantly different from the pair ratios of evoked release between 2 consecutive trials of stimulation at the same terminals (Mann-Whitney U-test, \(P = 0.15\)) but were significantly less than the average pair ratio of 2 random presynaptic terminals in a short axon segment (Mann-Whitney U-test, \(P = 0.02\)). D: the pair ratios for terminals innervating different dendrites from the same postsynaptic neuron were significantly higher than the pair ratios of 2 consecutive trials (Mann-Whitney U-test, \(P = 0.04\)) but were not significantly different from the pair ratio of 2 random presynaptic terminals (Mann-Whitney U-test, \(P = 0.13\)). E: the pair ratios for terminals innervating different postsynaptic neurons were significantly higher than the pair ratios of 2 consecutive trials (Mann-Whitney U-test, \(P = 0.002\)) but were not significantly different from the average pair ratio of 2 random presynaptic terminals (Mann-Whitney U-test, \(P = 0.314\)). The pair ratios of evoked release between 2 consecutive trials of stimulation at the same terminals were significantly less than the pair ratios of 2 random presynaptic terminals in a short axon segment (Mann-Whitney U-test, \(P < 0.001\)). Values are means \(\pm SE\); two-repeats control: 6 neurons, 49 terminals, and 49 pairs of trials; one axon, one dendrite: 9 neurons, 33 pairs of terminals; one axon, two dendrites: 8 neurons, 42 pairs of terminals; one axon, two neurons: 5 neurons, 30 pairs of terminals. Random pairs: 6 neurons, 261 pairs of terminals.

terminal area, from 0.3 to 8 \(\mu m^2\), was more accurately identified compared with simple thresholding. ROIs were defined using the cluster size range and a thresholded intensity, determined by measuring presynaptic terminals defined by eye in three representative images from the same set of images. These parameters were then used in all subsequent analyses.

Evoked release was measured by subtracting the total fluorescence in ROIs averaged from three consecutive frames right before the stimulation from the total fluorescence in ROIs in the one frame right after the stimulation was stopped. The total fluorescence in each ROI was determined by summing pixel intensity in each ROI. Among all ROIs, the fluorescence intensity of ROIs was plotted versus time;
some fluorescence traces increased linearly during stimulation, whereas others were flat (Fig. 1). The $R^2$ of the regression slope was calculated for each trace of fluorescence changes during stimulation for each ROI. The $R^2$ was also calculated for baseline fluorescence over 3.5 s preceding stimulation, assuming that during this period of time there is no evoked fluorescence change and any $R^2$ value is due to random fluorescence fluctuation. The distribution of $R^2$ values ($n = 1,387$ terminals) in the absence of stimulation was compared with the distribution of $R^2$ values during stimulation. As expected, during stimulation, the frequency of large $R^2$ values increased. To keep the rate of false positives low ($\leq 5\%$), $0.72$ was used as a threshold value. Thus a fluorescence trace with a positive slope during stimulation and with an $R^2$ value $>0.72$ was characterized as a releasing terminal. Those with an $R^2$ value $<0.72$ were regarded as nonreleasing terminals. These criteria match well with visual assessment of the change in fluorescence at presynaptic sites (c.f. Fig. 1B); for example, in Fig. 1B, terminal 4, which did not have a significant increase in fluorescence during stimulation, was defined as nonreleasing by these criteria. Unless stated otherwise, only presynaptic terminals that were characterized as releasing during 20-Hz stimulation were used in subsequent analyses.

Total vesicle pool size was estimated by subtracting the baseline fluorescence from the total fluorescence in NH$_4$Cl. Thus total vesicle pool size was measured as the maximum $\Delta F$ in NH$_4$Cl, as if all the vesicles clustered within a terminal were exocytosed. The release fraction of individual terminals was determined as $\Delta F$/total vesicle pool size and reflected the percentage of vesicles exocytosed on stimulation.

Colocalization between pHluorin$^+$ terminals and a pre- or postsynaptic marker after post hoc immunostaining was evaluated in time-lapse or immunostaining images taken at the same pixel resolution. The orientation of images after immunostaining was adjusted using free rotation in Metamorph, and the ROIs defined from time-lapse images were superimposed. The ROIs were moved together to align them with pHluorin$^+$ terminals. Individual ROIs were then moved by a few pixels for final alignment. If there was no pHluorin$^+$ terminal within 5 pixels of an ROI defined from time-lapse imaging, the ROI was not analyzed further. Different fluorescence channels were separated and segmented with the ImageJ algorithm as described in segmenting pHluorin$^+$ live images. An overlap mask of synaptophysin-pHluorin and an immunostained marker was made, ROIs were superimposed, and any pair of clusters with more than 1 pixel overlap within the ROI was defined as colocalized.

### Statistics

Data are means $\pm$ SE. The Kolmogorov-Smirnoff test was used to determine whether values were parametrically distributed. Student’s $t$-test was used for parametrically distributed values; the Mann-Whitney $U$-test or Wilcoxon matched-pairs test was used for nonparametrically distributed values; the Kruskal-Wallis ANOVA test was used for multiple group data comparison; the Pearson correlation test was used to compare correlated values; and the $F$-test was used to compare linear regressions (GraphPad Prism).

### RESULTS

**Evoked release is heterogeneous in short axon segments.**

Previous studies have shown that presynaptic terminals of cultured hippocampal neurons have heterogeneous release properties (Dobrunz and Stevens 1997; Hessler et al. 1993; Murthy et al. 1997; Rosenmund et al. 1993). However, the spatial arrangement of presynaptic release properties across long individual axons is poorly understood. To address this question, the evoked release of presynaptic terminals in $\sim 100$-$\mu$m segments of individual glutamatergic hippocampal neurons transfected with synaptophysin-pHluorin were first assessed by measuring the $\Delta F$ evoked by extracellular electrical stimulation, and then multiple segments along the entire axon arbor were assessed to map the overall distribution of presynaptic evoked release.

Axon segments with synaptophysin-pHluorin-labeled presynaptic terminals were selected for imaging on the basis of their presumptive apposition to postsynaptic dendrites, confirmed via post hoc immunostaining for the dendritic marker MAP2 (Fig. 1A, top). Stimulation at 20 Hz for 3.5 s with saturating current (MATERIALS AND METHODS) resulted in an increase in integrated $\Delta F$ ($\Delta F$ per pixel $\times$ area) at discrete sites along an $\sim 50$- to $100$-$\mu$m length of axon (Fig. 1, A and B). Stimulation-evoked $\Delta F$ was visible over time at most terminals along an axon segment (Fig. 1A, middle, terminals 1, 2, and 3). Vesicle clusters that were not responsive to electrical stimulation (Fig. 1A, terminal 4) may be trafficking packets of vesicles that are stationary during stimulation (Sabo et al. 2006) or immature, silent presynaptic terminals (Renger et al. 2001); these clusters were excluded from further analysis. As previously reported, $\Delta F$ of presynaptic terminals increased monotonically for the duration of the stimulus and then returned to baseline with a slower time course (Fig. 1B), as pHluorin was internalized and reacidified (Atluri and Ryan 2006; Burrone et al. 2006; Sankaranarayanan and Ryan 2000). Evoked release varied among terminals by more than 10-fold (Fig. 1). The local differences in evoked release among terminals along an axon segment did not follow any obvious spatial gradient.

To quantify the extent of presynaptic functional heterogeneity along $\sim 100$-$\mu$m segments, the random pair ratios of the evoked release between each possible pair of terminals in the field of view along the axon segment were calculated (to reduce random error, the higher value was always divided by a lower value). The higher the ratio, the greater the discrepancy or heterogeneity was between two terminals’ evoked release. The result from this measurement is consistent with the coefficient of variation (CV) measurements of presynaptic functional heterogeneity (Brancos et al. 2008), since the average pair ratios and CVs of the evoked release from the same set of presynaptic terminals are highly correlated (plot not shown, correlation test $P < 0.0001$). However, the pair ratio measurement enabled us to evaluate a relatively small number of terminals and define a reference value for relative “homogeneity” using the ratio of evoked release from two consecutive stimulations of the same terminal (at least 5 min apart). This two-repeats control ratio was useful to estimate the baseline variation due to trial-to-trial random variation so that any variation observed that was significantly higher than this value is due to other sources. The average control ratio was significantly less than the average ratio of evoked release between random pairs of presynaptic terminals (Fig. 1, C–E). Thus, although $\Delta F$ of a given terminal does vary somewhat with repeated stimulation, this source of variation alone does not account for the differences in $\Delta F$ between different terminals along short axon segments. This result is consistent with previous reports that evoked release of presynaptic terminals along an axon segment are heterogeneous. For subsequent analyses, a pair ratio higher than 2.6 (95% confidence interval of the average of the two-repeats ratio) was regarded as indicative of functional heterogeneity.

Next, we assessed whether presynaptic heterogeneity in evoked release is influenced by stimulus frequency. Previous work assessed heterogeneity in evoked release of presynaptic terminals using low-frequency pulse stimulation at 1 Hz or...
lower (Branco et al. 2008; Hessler et al. 1993; Huang and Stevens 1997; Murthy et al. 1997; Rosenmund et al. 1993). Because hippocampal neurons firing rates range from <2 to >100 Hz in awake rodents or during slow-wave sleep (Ranck 1973), evoked release was assessed across stimulation frequency. Terminals were stimulated with 70 action potentials delivered at 4 frequencies between 1 and 100 Hz. Among those stimulations, the 70 pulses delivered at 20 or 100 Hz mainly deplete the readily releasable pool of synaptic vesicles (Burrowe et al. 2006; Schikorski and Stevens 2001), whereas stimulation at 1 or 5 Hz allows time for the readily releasable pool vesicles to be refilled from the reserve pool and mainly reflects the release probability of individual terminals (Murthy et al. 1997). A 5-min interval between the different stimulation trains allowed the internalization and reacidification of surface synaptophysin-pHluorin. The heterogeneity in evoked release was similar across stimulation frequencies, as suggested by the evoked ΔF pair ratios for each stimulation frequency (pair ratios of evoked release: 1 Hz, 4.3 ± 0.9; 5 Hz, 4.3 ± 0.8; 20 Hz, 4.6 ± 0.9; 100 Hz, 4.9 ± 0.9; 1-way ANOVA, \(P = 0.13\)). This suggests that in short axon segments, the heterogeneity in postsynaptic strength is not influenced by brief stimulation over a wide frequency range.

Previous studies suggest that different postsynaptic targets, either different neurons or different dendrites of the same neuron, specify postsynaptic strengths (Branco et al. 2008; Koester and Johnston 2005). In contrast to previous studies, 20-Hz stimulation was used in our experiment to measure the local heterogeneity in evoked release, but not basal release probability, and how this depends on the synaptic configuration of terminals and postsynaptic neurons and/or dendrites. Pairs of presynaptic terminals in the same axon segment were classified as contacting the same dendrite (Fig. 1C), different dendrites from the same postsynaptic neurons (Fig. 1D), or different dendrites from different postsynaptic neurons (Fig. 1E). We found that the pair ratios of evoked release of terminals innervating two different dendrites from the same postsynaptic neuron (Fig. 1D) or two different neurons (Fig. 1E) were significantly higher than the two-repeats control. However, evoked release from terminals contacting the same dendrite was not significantly different from that of the two-repeats control (Fig. 1D). These results are consistent with previous studies measuring presynaptic strength using low-frequency stimulation (Branco et al. 2008; Koester and Johnston 2005). Together, these results suggest that the same axon can have terminals of different presynaptic strength, measured as evoked release during brief high-frequency stimulation, on different postsynaptic dendrites from the same or different neurons.

Presynaptic heterogeneity is correlated with the amount of postsynaptic NMDA receptors and PSD-95. Previous work showed that evoked release correlates with total vesicle pool size (Moulder et al. 2007). This correlation was verified by plotting the evoked ΔF against ΔF from NH4Cl (Fig. 2, A and B). A linear correlation between evoked ΔF and total vesicle pool size was observed (Fig. 2, A, 1st and 2nd panels, and B). Notably, terminals with larger total vesicle pool size showed larger evoked release than smaller terminals, and vice versa. These findings together suggest that the fraction of synaptic vesicles released (release fraction) during brief, high-frequency stimulation is similar among neighboring terminals over short axon distances.

To understand how postsynaptic targets may influence presynaptic evoked release, we assessed which of several postsynaptic constituents was correlated with presynaptic evoked release. Post hoc immunostaining was used to measure the amount of several postsynaptic proteins, including the NMDA receptor subunit NR1, the AMPA receptor subunit GluR1, and PSD-95 (colocalized pixel area × fluorescence intensity). MAP2 served as a negative control. Presynaptic terminals that were not colocalized with a postsynaptic dendrite, as identified by MAP2 immunostaining, or a postsynaptic protein cluster were excluded from this analysis.

Presynaptic terminals with higher evoked release (Fig. 2A, 1st panel) and larger total vesicle pool size (Fig. 2A, 2nd panel) were colocalized with larger NMDA receptor clusters (Fig. 2A, 3rd and 4th panels). In the example shown in Fig. 2A, a significant linear relationship was observed between presynaptic evoked release/total vesicle pool size and NMDA receptor clusters (Fig. 2, C and D) and in most segments (Fig. 2, E and F). A similar significant correlation was observed between presynaptic evoked release or total vesicle pool size and the amount of PSD-95 (Fig. 2, E and F). In contrast, the amount of GluR1 was not correlated with either evoked release or total vesicle pool size (Fig. 2, E and F). As expected, evoked release and total vesicle pool size were not significantly correlated with the amount of postsynaptic MAP2 in the vast majority of axon segments measured. These data show that presynaptic evoked release and total vesicle pool size are correlated with the amount of some, but not all, postsynaptic components.

Although these data suggest that the amount of postsynaptic NMDA receptors and PSD-95 may reflect presynaptic strength, neither protein is required for a presynaptic terminal to be functional. Of functional presynaptic terminals, 44 ± 9% were not colocalized with NMDA receptors and 36 ± 7% were not colocalized with PSD-95; 24% were not even colocalized with postsynaptic dendrites. This is consistent with previous work that showed that orphan presynaptic terminals without an apposed postsynaptic specialization were functional (Krueger et al. 2003). Nonetheless, over 7–14 days of maturation in vitro, total vesicle pool size did not increase in the population of presynaptic terminals that were not colocalized with PSD-95 (Fig. 2H); after 14 days in vitro, these terminals had significantly smaller total vesicle pool size than terminals colocalized with PSD-95 (Fig. 2, G and H). Together, these results suggest that NMDA receptor and PSD-95 are dispensable for the initial functionality of a presynaptic terminal, consistent with work in mice null for these proteins (Elias et al. 2006; Ulanir et al. 2007). However, these proteins accumulate postsynaptically as presynaptic terminals mature, in proportion to evoked release and total vesicle pool size.

Spatial distribution of functional properties of presynaptic terminals across individual axon arbor. How presynaptic functional properties varied across individual axon arbor was examined by cotransfecting neurons with mCherry to trace axon arbors for >1,000 µm (Fig. 3A) and sampling several 50- to 100-µm segments of axon over as much of the axon arbor as possible, always for more than several hundred micrometers. The distribution and mean of presynaptic properties was determined in different segments along an axon arbor. The
distribution was determined using the heterogeneity pair ratio measurement (Fig. 3), and the mean was determined by averaging the values of a particular functional property across axon segments (Fig. 4).

Within an axon segment, there is a wide range of pair ratios and thus heterogeneity in evoked release (Fig. 3B). For example, the pair ratios of evoked release for five axon segments from the neuron shown in Fig. 3A were significantly higher compared with the control ratios calculated from two consecutive stimulations (Fig. 3C). Evoked release was heterogeneous by this criterion in the majority (92%) of segments from each of six neurons assessed in this way. The pair ratios of evoked release of terminals with 20-Hz stimulation across each of six neurons assessed in this way. The pair ratios of total vesicle pool size were not significantly different for terminals in short axon segments compared with the entire axon arbor (e.g., Fig. 3E). Total vesicle pool size was similarly heterogeneous (Fig. 3D). Within each axon segment, the pair ratios of total vesicle pool size were significantly higher than the control ratios calculated from two consecutive measurements (Fig. 3E). Total vesicle pool size was heterogeneous by this criterion in the majority (75%) of segments from each of six neurons assessed in this way. The pair ratios of total vesicle pool size were not significantly different for terminals in short axon segments compared with the entire axon arbor (e.g., Fig. 3E), compare white bar with colored bars for 1 neuron; for all neurons, average of terminals in all short axon segments, 3.4 ± 0.2; average of all terminals in entire arbor, 3.4 ± 0.2; Mann-Whitney U-test, P = 0.41).

Total vesicle pool size was similarly heterogeneous (Fig. 3D). Within each axon segment, the pair ratios of total vesicle pool size were significantly higher than the control ratios calculated from two consecutive measurements (Fig. 3E). Total vesicle pool size was heterogeneous by this criterion in the majority (75%) of segments from each of six neurons assessed in this way. The pair ratios of total vesicle pool size were not significantly different for terminals in short axon segments compared with the entire axon arbor (e.g., Fig. 3E), compare white bar with colored bars for 1 neuron; for all neurons, average of terminals in all short axon segments, 3.2 ± 0.2; average of all terminals in entire arbor, 3.6 ± 0.3; Mann-Whitney U-test, P = 0.24).

To further investigate how the heterogeneity in total vesicle pool size was related to the heterogeneity in evoked release proximally and distally, both CV and pair ratio

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**Fig. 2.** Presynaptic heterogeneity is correlated with the amount of postsynaptic NMDA receptors and PSD-95. Local axon segments were stimulated with 70 pulses delivered at 20 Hz to measure evoked release, followed by NH4Cl saline perfusion to measure total vesicle pool sizes. Post hoc immunostaining was performed with PSD-95, NR1, GluR1, or MAP2 antibodies to reveal postsynaptic specializations or dendrites. We examined 6–8 neurons for each marker. A: representative images of evoked ΔF (1st panel), total vesicle pool size (2nd panel), post hoc immunostaining of NR1 clusters (3rd panel; green channel is pHluorin, red channel is NR1), and colocalized pixels between NR1 and synaptophysin-pHluorin (4th panel). Arrowheads indicate the terminals plotted in B and C. Scale bar, 5 μm. B: correlation plot of total vesicle pool size and evoked ΔF of terminals shown in A (correlation coefficient r = 0.98, Pearson correlation test, P = 0.02). C: correlation plot of NR1 intensity and evoked ΔF of terminals shown in A (r = 0.99, Pearson correlation test, P = 0.006). D: correlation plot of NR1 intensity and total vesicle pool size of terminals shown in A (r = 0.97, Pearson correlation test, P = 0.03). E: the average correlation coefficient between evoked release and NR1 (9 segments; r = 0.8 ± 0.04) or PSD-95 (11 segments; r = 0.80 ± 0.04) was significantly different from the MAP2 correlation coefficient (Mann-Whitney U-test; NR1, *P = 0.003; PSD-95, *P = 0.003) but not that of GluR1 (r = 0.6 ± 0.1, P = 0.27). Evoked release was not significantly correlated with the amount of MAP2 for terminals in 7/8 axon segments measured (r = 0.4 ± 0.1). F: the average correlation coefficients between total vesicle pool size and NR1 (r = 0.8 ± 0.03) or PSD-95 (r = 0.80 ± 0.04) were significantly different from the MAP2 correlation coefficient (Mann-Whitney U-test; NR1, *P = 0.008; PSD-95, *P = 0.01) but not that of GluR1 (r = 0.7 ± 0.1, P = 0.17). Total vesicle pool size was not significantly correlated with the amount of MAP2 for terminals in 6/8 segments measured (r = 0.5 ± 0.1). G: cumulative distribution of total vesicle pool size in presynaptic terminals colocalized with PSD-95 increased significantly from 10 to 21 DIV (Kruskal-Wallis test, *P < 0.02). H: cumulative distribution of total vesicle pool size in presynaptic terminals not colocalized with PSD-95 was not significantly different from 10 to 21 DIV (Kruskal-Wallis test, P = 0.13). Cc, correlation coefficient.
values, which are size-independent measures of heterogeneity, were used. A significant linear correlation was observed between the CV of total vesicle pool size and the CV of evoked release (Fig. 3F). A similar result was obtained by using the pair ratio as a measure of heterogeneity (plot not shown; correlation test, $P < 0.0001$). This result suggests that although the distribution of evoked release and total vesicle pool size is similar, the heterogeneity in total vesicle pool size underlies the heterogeneity in evoked release, regardless of release fraction.

**Evoked release and release fraction are higher in distal segments of individual axon arbors.** The average value of evoked release and total vesicle pool size in short axon segments were determined and were compared from the most proximal and most distal axon segments. Distal axon segments had a significantly higher evoked release than proximal segments (1-way ANOVA, $P = 0.92$). F: relationship between coefficient of variance (CV) of evoked release and total vesicle pool size of presynaptic terminals from the most proximal (white circles; $r = 0.65$, Pearson correlation test, $P < 0.05$) and the most distal segments (black circles; $r = 0.82$; Pearson correlation test, $P < 0.05$) from 13 neurons (slope = $0.96 \pm 0.05$; Pearson correlation test, $P < 0.0001$).

Since evoked release correlates with total vesicle pool size in short axon segments such that release fraction is constant, how can this observation be reconciled with the observation that terminals in distal axon segments have a higher release fraction than proximal segments (Fig. 4B). Thus distal axon segments had a significant, 2.1-fold higher release fraction than proximal segments (Fig. 4C).

Since evoked release correlates with total vesicle pool size in short axon segments such that release fraction is constant, how can this observation be reconciled with the observation that terminals in distal axon segments have a higher release fraction? By plotting evoked release against total vesicle pool size, a strong linear relationship was observed between these two properties in most short axon segments, regardless of their location in the axon arbor (Fig. 4D, data correspond to color-
Fig. 4. Evoked release and release fraction are higher in distal compared with proximal segments of individual axon arbors. A: average evoked release in proximal axon segments (black symbols; 1.3 ± 0.2 × 10^4 AFU) is slightly but significantly smaller than distal segments (1.9 ± 0.3 × 10^4 AFU; 16 neurons; Wilcoxon matched-pairs test, P < 0.001). The average evoked release in proximal compared with distal segments is indicated by red symbols. B: average total vesicle pool size in proximal axon segments (black symbols; 15.2 ± 3.9 × 10^4 AFU) of individual neurons is not significantly different from that in distal segments (10.1 ± 2.0 × 10^4 AFU; 16 neurons; Wilcoxon matched-pairs test, P = 0.17). The average of total vesicle pool size in proximal compared with distal segments is indicated by red symbols. C: average release fraction in proximal axon segments (black symbols; 0.15 ± 0.02) is significantly smaller than that in distal segments (0.30 ± 0.04; 16 neurons; Wilcoxon matched-pairs test, P = 0.003). The average release fraction of proximal compared with distal segments is indicated by red symbols. D: relationship between evoked release and total vesicle pool size of presynaptic terminals from axon segments I-V from the representative neuron shown and color coded in Fig. 3A. Correlation coefficient and Pearson correlation test results for each segment: segment I, r = 0.67, P = 0.15; segment II, r = 0.86, P = 0.006; segment III, r = 0.81, P = 0.002; segment IV, not available; segment V, r = 0.88, P = 0.004. In this example, as for all axons measured (24 segments, 6 neurons), some segments have a similar regression slope, indicative of similar release fraction (segments I and II, segments III and IV), whereas release fraction is significantly different among other segments (1-way ANOVA, P = 0.007). E: relationship between evoked release and total vesicle pool size of all terminals in the most proximal segments (gray) and the most distal segments (black) from 13 neurons (evoked release: r = 0.90, Spearman correlation test, P < 0.0001; total vesicle pool size: r = 0.85, Spearman correlation test, P < 0.0001). The linear regression lines are significantly different (average proximal slope = 0.05 ± 0.005, distal slope = 0.14 ± 0.009; F-test, P < 0.001), indicating that distal terminals have a significantly higher release fraction compared with proximal terminals.

Although most terminals measured were apposed to postsynaptic dendrites, the release fraction of terminals not apposed to dendrites was not significantly different from those that were (5 of 6 neurons; Student’s t-test, P > 0.05; data not shown). This is consistent with the observation that local release fraction is relatively constant regardless of the spatial configuration of postsynaptic targets. These data indicate that release fraction is not well correlated with postsynaptic targets.

Thus, in contrast to the correlation between evoked release and total vesicle pool size in short axon segments, evoked release and thus terminal release probability is modulated differently across the entire axon arbor. This is because release fraction, but not total vesicle pool size, accounts for higher evoked release distally compared with proximally.

DISCUSSION

Here we report the distribution of the functional properties of presynaptic terminals and their relationship with postsynaptic specializations across two spatial scales. Over short axon arbor distances (~100 μm), evoked release as measured during brief 20-Hz stimulation was spatially heterogeneous for terminals contacting dendrites on the same/different postsynaptic
neurons but not for terminals contacting the same dendrites. The evoked release and total vesicle pool size spatially covaried, regardless of the spatial configuration of the postsynaptic target(s). This suggests that the fraction of synaptic vesicles released is similar among neighboring terminals over short axon distances. Evoked release and total vesicle pool size were highly correlated with the amount of two postsynaptic proteins, NMDA receptors and PSD-95, regardless of the spatial configuration of postsynaptic targets. Our results suggest that local presynaptic strength is determined mainly by local interactions with postsynaptic targets, correlated with modulation of total vesicle pool size, associated with the amount of postsynaptic NMDA receptors and PSD-95.

We report for the first time that over long distances (~1,000 μm) across the arbor of single axons, a proximal-to-distal difference exists in evoked release. In contrast to short axon segments, distal terminals have higher evoked release, but not total vesicle pool size, than proximal terminals. Thus release fraction is increased in distal terminals, likely due to greater release probability of individual vesicles and/or readily releasable pool size distally compared with proximally. Our results suggest that across the arbor of a single axon, the mechanisms that determine synaptic strength are different, depending on spatial scale.

Retrograde cellular and molecular determinants of heterogeneous presynaptic release properties in short axon segments. Previous work has suggested that postsynaptic targets retrogradely influence presynaptic release probability (Branco and Staras 2009; Branco et al. 2008; Koester and Johnston 2005; Reyes et al. 1998). In rat neocortex, presynaptic terminals contacting different postsynaptic neurons have heterogeneous release probability (Koester and Johnston 2005; Reyes et al. 1998), consistent with the results we report here. In contrast, presynaptic terminals contacting different dendrites of the same postsynaptic neuron have been reported to have heterogeneous release probability in cultured rat hippocampal neurons (Branco et al. 2008). Our experiment were mainly carried out with 20-Hz stimulation, a physiologically relevant stimulus for hippocampal neurons (Waters and Smith, 2002). We did not directly assess “basal” release probability, but instead measured the vesicles immediately available for release (Burrone et al. 2006; Pyle et al. 2000; Schikorski and Stevens 2001; Waters and Smith, 2002), which will depend on vesicle release probability and any contribution of vesicle replenishment during higher frequency stimulation. Similar to previous studies (Branco et al. 2008), our results suggest that there is heterogeneity among terminals of the same axon contacting different dendrites, but not among terminals of an axon contacting the same dendrite.

Several different postsynaptic retrograde mechanisms have been suggested to influence presynaptic functional properties (Dalva et al. 2007; De Paola et al. 2003; Pratt et al. 2003; Tao and Poo 2001; Williams et al. 2010; Ziv and Garner 2004). These include cell adhesion molecules such as the neuroligin-neurexin complex (Chih et al. 2005; Futai et al. 2007; Levinson et al. 2005), SynCAM (Biederer et al. 2002), EphBs and ephrin-Bs (Dalva et al. 2000; Kayser et al. 2006), and cadherins (Arikkath and Reichardt 1998; Benson and Tanaka 1998; Takeichi 2007), among others, as well as secreted factors such as neurotrophins (Du and Poo 2004), FGF22, FG7 (Terauchi et al. 2010), and Sema3F (Tran et al. 2009), among others. Which of these cell-cell signaling mechanisms contribute to the modulation of presynaptic release properties on different spatial scales across an axon arm remains to be determined.

Here we show that the amount of synaptic NMDA receptors and PSD-95 is highly correlated with presynaptic strength measured as evoked release, regardless of the postsynaptic neuron target(s). However, it is unlikely that NMDA receptors or PSD-95 directly specify presynaptic strength. Synapse formation and function are largely normal in the absence of PSD-95, probably due to functional redundancy (Elias et al. 2006). However, neuroligins that bind to the PDZ domain of PSD-95 have been shown to retrogradely modulate presynaptic release probability (Futai et al. 2007; Wittenmayer et al. 2009). This suggests that PSD-95 correlates with presynaptic strength indirectly, possibly via neuroligin-1 signaling. Although blocking NMDA receptor currents does not change the distribution of other pre- and postsynaptic molecules, except for a homeostatic increase in NMDA receptors (Rao and Craig 1997), deletion of NMDA receptors in cortical pyramidal neurons resulted in fewer but larger spines and presynaptic terminals (Ultanir et al. 2007). The correlation between NMDA receptors and presynaptic strength we observed may be because NMDA receptors bind the first PDZ domain of PSD-95 (Irie et al. 1997), which through its interactions with neuroligins retrogradely modulates release probability, resulting in a correlation between the amount of PSD-95 and presynaptic strength. Other adhesion and signaling molecules associated with NMDA receptors or PSD-95, such as EphBs (Dalva et al. 2000; Kayser et al. 2006), may also retrogradely modulate synaptic strength. The observation that the amount of GluR1 does not correlate with presynaptic strength, whereas the amount of NMDA receptors and PSD-95 does, suggests that not all postsynaptic proteins are affected by retrograde signaling that matches pre- and postsynaptic properties. Recent studies showed that the amount of AMPA receptors does not correlate with presynaptic strength with baseline activity (Tokuoka and Goda 2008), and AMPA receptor subunits retrogradely stabilize presynaptic terminals when neuroligin-1 is present (Ripley et al. 2010). Our work suggests that the amount of two postsynaptic proteins, NMDA receptors and PSD-95, is an indirect indicator of presynaptic strength, regardless of the spatial configuration or the identity of postsynaptic neuron targets.

Spatial distribution of heterogeneous presynaptic terminals across axon arbors. Different spatial patterns of presynaptic strength have been documented for axons and terminals in several different model systems. Heterogeneity in the strength of presynaptic evoked release has been observed along CNS axons segments and within individual mouse motor nerve terminals, whereas proximal-to-distal gradients of exocytosis have been observed in motor nerve terminals of fly, toad, and crayfish (Atwood 1967; Bennett et al. 1986; Guerrero et al. 2005). Whether large-scale spatial patterns of presynaptic strength also exist across the arbor of CNS axons has not been well studied prior to the work we present here.

Consistent with previous work, we found that in short axon segments, presynaptic terminals were heterogeneous with respect to evoked release and total vesicle pool size, over an approximately threefold range of values (Branco et al. 2008; Hessler et al. 1993; Moulder et al. 2007; Murthy et al. 1997; Rosenmund et al. 1993). In short axon segments, evoked
release was correlated with total vesicle pool size, similar to other studies using pHluorin (Moulder et al. 2007) and electron microscopy studies that showed large presynaptic terminals contain more vesicles (Dobrunz and Stevens 1997; Rosenmund and Stevens 1996; Schikorski and Stevens 2001). Since our stimulation parameters (20 Hz, 70 pulses) were chosen to deplete the readily releasable pool of synaptic vesicles (Burron et al. 2006; Pyle et al. 2000; Schikorski and Stevens 2001; Waters and Smith, 2002), the evoked release measured with this stimulation paradigm mainly reflects the size of the readily releasable pool in the absence of vesicle replenishment (Leitz and Kavalali 2011; Zhu et al. 2009). The magnitude of evoked release can be predicted by a simple model that takes into account the size of the readily releasable pool size, the release probability of individual vesicles, and the number of times the terminal is stimulated (Fig. 5A). For short axon segments, this model also mimics the dependence of the heterogeneity in evoked release on total vesicle pool size, if the readily releasable pool size is constrained to scale in proportion to the total vesicle pool size (Fig. 5B; note linear relationship for either proximal or distal segments). Thus, in short axon segments, the heterogeneity in evoked release can be explained by the heterogeneity in total vesicle pool size, independent of stimulation frequency. This may be important for maintaining the relative strength of terminals across a range of action potential firing rates, similar to those present in vivo.

We report here for the first time that evoked release was significantly higher, by 1.5-fold, in terminals located in proximal compared with distal axon segments. In contrast to the heterogeneity in short axon segments, in which terminals with larger total vesicle pool size have higher evoked release, the proximal low/distal high evoked release was not correlated with total vesicle pool size, because the distal segments that had higher evoked release had the same, or smaller, average total vesicle pool size. The distal increase in evoked release and release fraction is also predicted by the simple model, given either an increase in individual vesicle release probability and or an increase in readily releasable pool size, if the readily releasable pool is increased independently of total vesicle pool size (Fig. 5B; compare change in slope of linear relationship for proximal vs. distal segments). The postulated presynaptic loci for the distal increase in release properties is consistent with our observation that these differences are not correlated with the spatial configuration of postsynaptic targets or the amount of postsynaptic components. However, our results cannot exclude the possibility that the proposed changes in presynaptic properties are secondary to contributions from additional attributes of the postsynaptic neuron that were not assessed in this study. The spatial configuration of presynaptic axon interactions with postsynaptic targets is obviously different in vitro compared with in vivo. Thus measuring the spatial distribution of functional properties in presynaptic terminals in vivo is an important future direction. A previously reported mechanism that could increase distal release by increasing individual vesicle release probability is found in Drosophila motor nerve terminals, where higher distal release is coupled to higher Ca\(^{2+}\) influx (Guerrero et al. 2005). Conversely, in crayfish, higher distal release is correlated with boutons that have denser T-bars within active zones (Atwood 1967), suggestive of a larger readily releasable pool. Future studies employing ultrastructural studies of the number of docked vesicles in distal compared with proximal terminals, and measurements of Ca\(^{2+}\) influx in individual presynaptic terminals to assay the sensitivity of the release fraction to external Ca\(^{2+}\), would provide further insights into the prominent proximal-to-distal difference in evoked release across an individual axon arbor.

Fig. 5. Higher distal compared with proximal evoked release can be accounted for by higher distal individual vesicle release probability and/or readily releasable pool size. A simple model of vesicle exocytosis in hippocampal nerve terminals was capable of recapitulating the experimental data and provides some insight in the possible mechanisms underlying spatial differences in release properties. A: the number of vesicles releasing neurotransmitter (ER, evoked release) was given by the number of vesicles in the readily releasable pool (RRP), the release probability of individual vesicles (Pv), and the number of times the axon was stimulated (N). The size of the RRP was proportional to total vesicle pool size (TVP), defined as the sum of the RRP, the reserve pool, and the resting pool. The fraction of vesicles released (RF) was defined as the ratio of ER to TVP. Thus ER = RRP \times PV \times N; RRP = k \times TVP; and RF = ER/TVP, where k = RRP/TVP. Based on an anatomical model of vesicle distribution in terminals (Südhof 2000), TVP was set at 200 vesicles and RRP at 5 vesicles, which yields an initial value of k = 0.025. After substitution and rearrangement, individual vesicle release probability is Pv = RF/(k \times N); the measured value of RF, 0.15 for proximal segments, can be used to determine an initial value for Pv in the model. B: TVP was systematically varied over a 10-fold range to generate heterogeneity in TVP and yield values of ER consistent with the experimental data from proximal segments (black line). The measured increase in RF for distal segments from 0.15 to 0.30 could be achieved in the model by either doubling the Pv (red line) or increasing the size of the RRP independent of the size of the TVP, i.e., doubling k (blue line). This holds true for any combination of Pv and RRP such that Pv \times k = RF/N. These model findings are consistent with the notion that differences in ER within short axon segments (either proximal or distal) can be attributed to the changes in terminal size, or at least TVP size, whereas proximal/distal differences arise from either changes in RRP size or Pv.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: X.P., T.D.P., and R.J.B.-G. conception and design of research; X.P. performed experiments; X.P. and T.D.P. analyzed data; X.P. interpreted results of experiments; X.P. prepared figures; X.P. drafted manuscript; T.D.P. and R.J.B.-G. edited and revised manuscript; R.J.B.-G. approved final version of manuscript.

REFERENCES


