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Fusion pore flux controls the rise-times of quantal synaptic responses

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The release of neurotransmitter from a single synaptic vesicle generates a quantal response, which at excitatory synapses in voltage-clamped neurons is referred to as a miniature excitatory postsynaptic current (mEPSC). We analyzed mEPSCs in cultured mouse hippocampal neurons and in HEK cells expressing postsynaptic proteins enabling them to receive synaptic inputs from cocultured neurons. mEPSC amplitudes and rise-times varied widely within and between cells. In neurons, mEPSCs with larger amplitudes had longer rise-times, and this correlation was stronger in neurons with longer mean rise-times. In HEK cells, this correlation was weak and unclear. Standard mechanisms thought to govern mEPSCs cannot account for these results. We therefore developed models to simulate mEPSCs and assess their dependence on different factors. Modeling indicated that longer diffusion times for transmitters released by larger vesicles to reach more distal receptors cannot account for the correlation between rise-time and amplitude. By contrast, incorporating the vesicle size dependence of fusion pore expulsion time recapitulated experimental results well. Larger vesicles produce mEPSCs with larger amplitudes and also take more time to lose their content. Thus, fusion pore flux directly contributes to mEPSC rise-time. Variations in fusion pores account for differences among neurons, between neurons and HEK cells, and the correlation between rise-time and the slope of rise-time versus amplitude plots. Plots of mEPSC amplitude versus rise-time are sensitive to otherwise inaccessible properties of a synapse and offer investigators a means of assessing the role of fusion pores in synaptic release.

Introduction

Neurotransmitter is released from a synaptic vesicle into the synaptic cleft. After passing through a fusion pore, the transmitter diffuses and activates postsynaptic receptors to generate a post-synaptic response. The spontaneous release of a single vesicle at an excitatory synapse on a voltage-clamped cell produces a miniature excitatory postsynaptic current (mEPSC). This represents the basic quantal unit of synaptic transmission. Studies of synaptic transmission often focus on mEPSCs in efforts to probe basic mechanisms. A general obstacle to such efforts is that mEPSCs vary widely and are sensitive to many factors (Finch et al., 1990; Redman, 1990; Bekkers, 1994). Dendritic location (Bekkers and Stevens, 1996), stochastic channel gating (Faber et al., 1992; Raghavachari and Lisman, 2004), vesicle size (Bekkers et al., 1990; Karunanithi et al., 2002; Guo et al., 2015), cleft transmitter concentration (Liu et al., 1999; Franks et al., 2003), fusion pore kinetics (Guo et al., 2015), and release site-receptor registration (Uteshev and Pennefather, 1996; MacGillavry et al., 2013) have all been implicated or hypothesized to influence mEPSC shape and variability. The compact geometry of synapses minimizes diffusion times to

maximize speed (Eccles and Jaeger, 1958; Biederer et al., 2017). The combined action of many rapid processes makes it difficult to evaluate specific roles and determine how these many factors shape mEPSCs.

To address this issue, the present study focused on a positive correlation between mEPSC amplitude and rise-time. This form of correlation has been reported previously in the neuromuscular junction (Negrete et al., 1972; Hartzell et al., 1975; Wathey et al., 1979), cerebellar granule cells (Wall and Usowicz, 1998), hippocampal pyramidal cells (Magee and Cook, 2000), inhibitory synapses in cultured hippocampus (Barberis et al., 2004), and calyx of Held (Guo et al., 2015). In the present study, we analyzed our own published data from cultured hippocampal neurons (Chiang et al., 2021) and reported this form of correlation: mEPSCs with larger amplitudes tend to have longer rise-times. Data from two different types of synapses were compared: neuron–neuron synapses and heterologous synapses formed by neurons with HEK cells expressing postsynaptic proteins (Chiang et al., 2021). In neurons, the correlation was very clear and was preserved at the single-cell level. A new

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correlation was found in single-cell data between the mean rise-time and the slope of the plot of rise-time versus amplitude. By contrast, in HEK cells cocultured with neurons mEPSC rise-times and amplitudes had little if any correlation.

A positive correlation between amplitudes and rise-times of mEPSCs presents a serious challenge to interpretation. The potential sources of variation noted above generally predict that increasing amplitude should either increase the rate of rise or leave it unchanged. Negrete et al. (1972) suggested a mechanism based on synaptic cleft diffusion. A large vesicle releases more neurotransmitter to produce a large amplitude event and saturate proximal receptors. The excess transmitter is then free to diffuse to distal receptors to extend the rise-time. Another possible explanation not previously considered builds on the notion of exponential loss of transmitter through a static fusion pore (Almers et al., 1991). For uniform pores with a fixed permeability, larger vesicles release neurotransmitters over a longer time to extend the mEPSC rise-time. To evaluate hypothetical mechanisms in the control of mEPSCs, we developed models that combine fusion pore flux, cleft diffusion, and receptor activation. We explored how vesicle content, receptor density, synapse size, fusion pore permeability, and diffusion coefficient interact to shape mEPSCs. Model simulations indicated that diffusion makes a very small contribution to the correlation between amplitude and rise-time. By contrast, slower content loss through a fusion pore extended the rise-time for EPSCs produced by larger vesicles. A model incorporating this feature robustly recapitulated experimental behavior and provided insight into the interplay between fusion pore flux and receptor activation in shaping mEPSCs.

Materials and methods

Experimental

Data analyzed in the present study were from a previous report, where a full account of experimental methods can be found (Chiang et al., 2021). mEPSCs were recorded with an Axopatch 200B amplifier using Sylgard-coated patch electrodes. Series resistances were low and well compensated to ensure accurate measurements of rise-times. Recordings were made from cells bathing in a solution consisting of (in mM) 140 NaCl, 5 KCl, 5 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose, pH 7.4, at room temperature. Spontaneous mEPSCs were recorded under voltage clamp at -65 mV in the presence of tetrodotoxin to block action potentials, aminophosphonovaleric acid to block NMDA receptors, and SR95531 to block GABA_A receptors. Recordings of neuron–neuron mEPSCs were made in mouse hippocampal cultures at 18 days. Recordings of neuron–HEK cell mEPSCs were made from HEK293T cells 1 day after adding them to 17-day hippocampal cultures. On the day prior to addition, the HEK cells were transiently transfected with four postsynaptic proteins, GluA2 AMPA-type glutamate receptors, PSD-95, neuroligin 1, and stargazin. mEPSCs were analyzed using in-house software that fits events to a function with an exponential rise and decay (see Eq. 1 and Fig. 2 A of Chiang et al. [2021]). Peak amplitude, 10–90% rise-time, and area were calculated for each mEPSC from the parameters of the fit.

Table 1. Amplitude, area, and rise-time (mean ± SE) of mEPSCs recorded from neurons and HEK cells (error based on cell number, in parentheses)

	Amplitude pA	Area pA- msec (fC)	10–90 rise-time msec	Event number
Neurons (29)	-19.7 ± 1.1	98.0 ± 7.9	0.663 ± 0.043	3,170
HEK cells (24)	-32.8 ± 3.6	81.5 ± 10.2	0.332 ± 0.017	2,715

Data from Chiang et al. (2021); see Fig. 2 of that paper.

Statistical analysis

Analysis was performed with the computer program Origin 2023b. Linear regression was used to evaluate correlations and determine P values. ANOVA was used to evaluate variation between cells. The Kolmogorov–Smirnov test was used to test for normality in data from single cells. Errors are SE of the mean using mEPSC number from each cell in Fig. 3, and SE of the mean using cell number in Table 1. The data consists of 3,171 neuron–neuron mEPSCs recorded from 29 neurons in neuron-only cultures, and 2,716 neuron–HEK cell mEPSCs recorded from 24 HEK cells in cocultures (Chiang et al., 2021).

Results

Correlations between mEPSC rise-time and amplitude

Fig. 1 presents sample traces of mEPSCs recordings from neurons and HEK cells. Long-duration traces illustrate the trend of higher frequency in HEK cells and short-duration traces illustrate the scatter and overall higher amplitudes in HEK cells. The mean amplitude, rise-time, and area are presented here in Table 1. They were presented in graphic form as Fig. 2 of Chiang et al. (2021). Amplitudes in Table 1 can be seen to be 66% larger in HEK cells and rise-times were about half that in neurons. The mean areas are similar suggesting similar average neurotransmitter content in the vesicles of both synapses.

The traces in Fig. 1 illustrate the large variability in mEPSCs. Distributions and variance in amplitude and area are widely used to study synaptic function. Going beyond distributions, joint variations can also be informative and the features of mEPSCs can be plotted against one another to evaluate correlations. This offers a strategy for probing mechanisms because, as will be examined in depth here, different models for mEPSCs predict characteristic forms of correlations. Here, we focus on amplitude and rise-time. Rise-times and amplitudes of 3,171 mEPSCs from 29 neurons and 2,716 mEPSCs from 24 HEK cells were plotted as individual points in Fig. 2, A and B. Linear regression indicated highly significant correlations. The slope for neurons was 0.0077 ± 0.0006 msec/pA and for HEK cells was 0.00065 ± 0.00011 msec/pA (P < 0.0001 for both). Previous studies of scatter plots such as those displayed here in Fig. 2, A and B, reported significant correlations. The slopes were 0.0017 msec/pA in calyx of Held (Guo et al., 2015) and 0.006 msec/pA in hippocampal pyramidal cells (Magee and Cook, 2000).

With so many points, visual assessment is difficult, so we binned the data. These binned plots illustrate the correlations

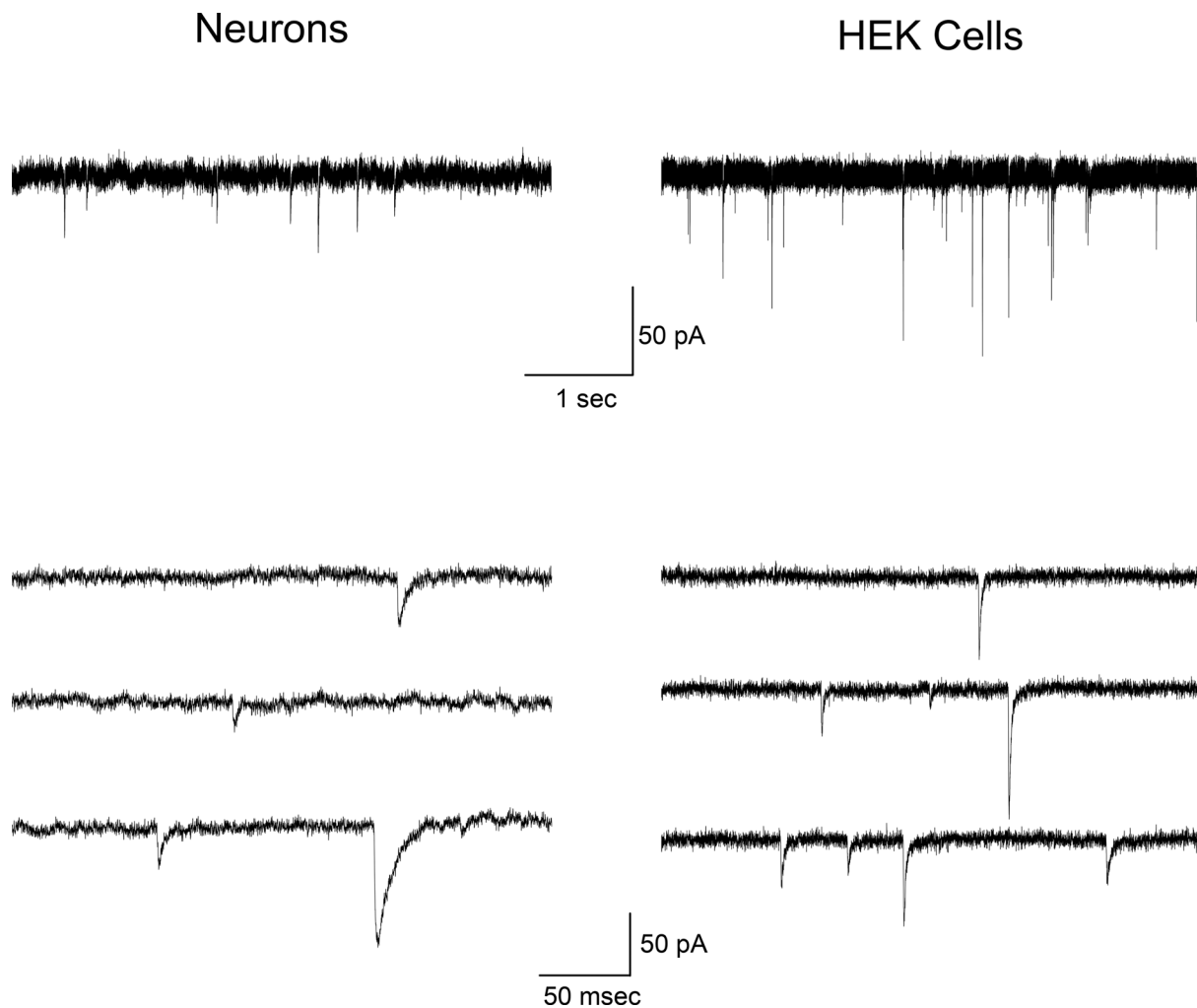


Figure 1. **mEPSC recordings.** mEPSCs recorded from neurons (left), and from HEK cells (right) expressing postsynaptic proteins (GluA2, neuroligin 1, PSD-95, and stargazin) and co-cultured with neurons. Traces were drawn from the data of Chiang et al. (2021). Long-time traces above illustrate the overall incidence. Short-time traces below illustrate the varied shapes of individual events.

more clearly (Fig. 2, C and D). The plot for neurons has a clear positive slope of 0.0089 ± 0.0007 msec/pA. The plot for HEK cells has a much smaller positive slope of 0.00060 ± 0.00011 msec/pA that is nevertheless significantly larger than zero. The pooled and binned plots produced qualitatively similar results.

Fig. 2 pooled the data from many cells. This raises the question of whether the correlation is uniform across cells and synapses. Furthermore, quantities could co-vary. If some cells or synapses have both larger amplitudes and longer rise-times while other cells or synapses have smaller values for both, then the appearance of a correlation in the pooled data would reflect synapse heterogeneity rather than fundamental dynamic processes within synapses. We therefore averaged amplitude and rise-time in each of the 29 neurons and 24 HEK cells. These quantities varied dramatically between cells, with ANOVA yielding $P < 0.0001$ for the hypothesis that values are drawn from a uniform distribution. Plotting these cell averages illustrates this variation (Fig. 3). The SE of individual points is very small compared with the ranges spanned. Furthermore, linear regression indicated no significant correlations between the cell

averages of amplitude and rise-time in either plot. Thus, although mEPSC amplitude and rise-time vary widely between cells, they do not co-vary. The correlations in Fig. 2 therefore do not arise from co-variation of rise-time and amplitude between cells (Fig. 3).

The variations in Fig. 3 indicate that factors shaping mEPSCs differ widely between cells. Furthermore, the variations form an interesting pattern. In neurons, the rise-times are more variable than the amplitudes. In HEK cells, we see the reverse: the rise-times fall in a narrow range and the amplitudes are much more spread out. Thus, we see a quasi-orthogonal relation between the patterns of variation between the two conditions: in neurons the vertical variation (in rise-time) dominates and in HEK cells the horizontal variation (in amplitude) dominates. This suggests different sources of variability between the mEPSCs of neurons and HEK cells, and this will be explored further below.

Fig. 3 provides a clear rejection of covariation between cells. It is more difficult to address the question of whether amplitudes and rise-times co-vary between individual synapses in the same cell. A single neuron has multiple synapses that

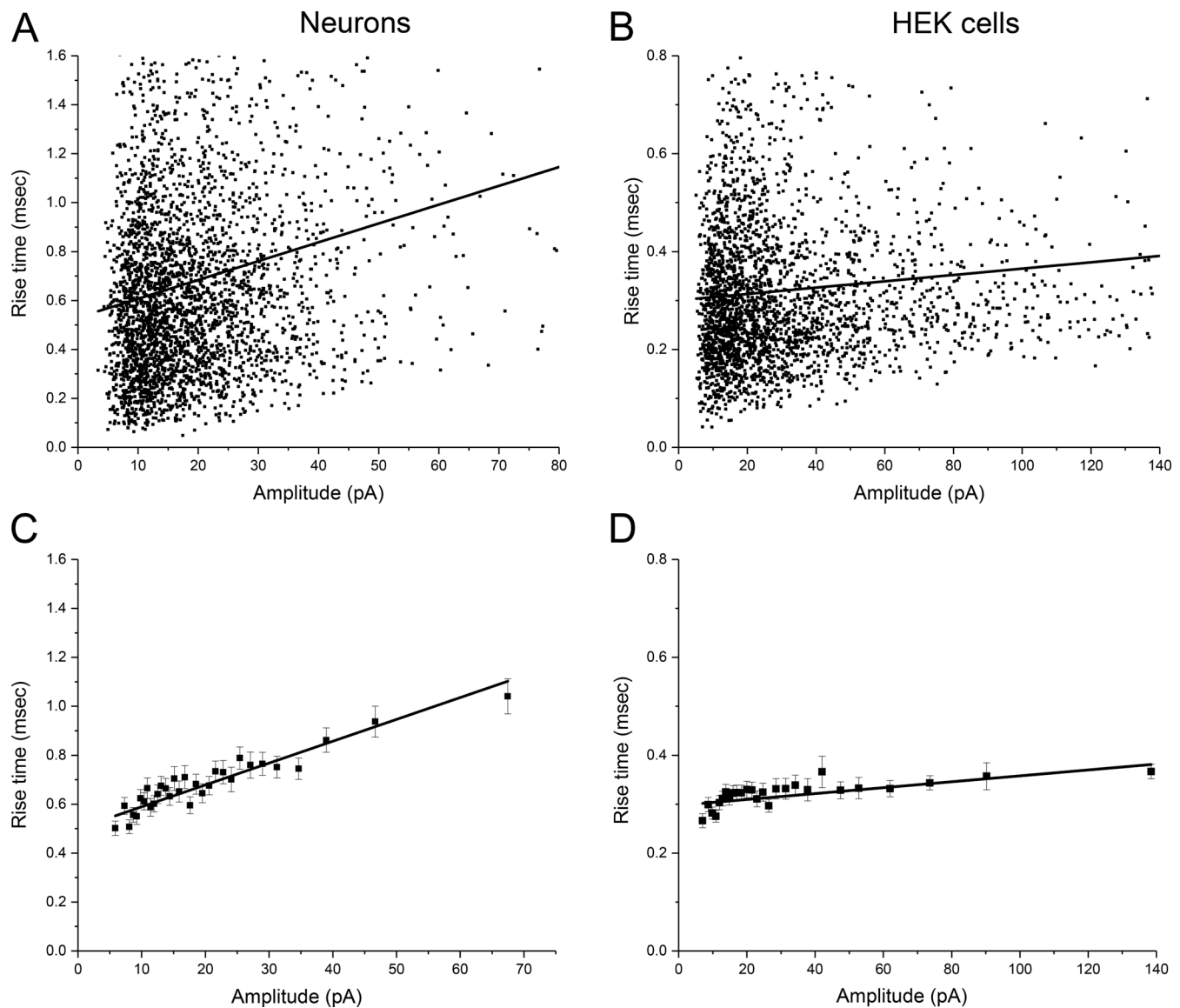


Figure 2. **Plots of mEPSC rise-time versus amplitude.** Scatter plots with each point reflecting one mEPSC. **(A)** A plot of 3,170 points from neurons; linear regression yielded a slope of 0.0077 ± 0.0006 msec/pA ($P < 0.0001$); 135 points had rise-times outside the range >1.6 msec. **(B)** A plot of 2,715 points from HEK cells had a slope 0.00065 ± 0.00011 msec/pA ($P < 0.0001$); 48 points had rise-times >0.8 msec. These points were then binned in groups of 50 along the amplitude axis. **(C)** Binned plot for neurons has a slope 0.0089 ± 0.0007 msec/pA. **(D)** Binned plot for HEK cells has a slope 0.00060 ± 0.00011 msec/pA. The slopes were much steeper for neuron–neuron synapses than for neuron–HEK cell synapses.

are heterogeneous in their shape as well as their release probability (Bekkers and Stevens, 1995; Ariel et al., 2013; Ralowicz et al., 2024). In the present study, the average number of mEPSCs recorded per cell was 109 for neurons and 113 for HEK cells, and these numbers are adequate to use the Kolmogorov–Smirnov test of the hypothesis of a normal distribution. For neurons 9/29 cells had amplitudes and 23/29 cells had rise-times consistent with a normal distribution ($P > 0.05$). For HEK cells, the respective numbers were 8/24 and 15/24. Furthermore, both quantities satisfied the test of normality in 6/29 neurons and 4/24 HEK cells. Of the six neurons passing in both quantities, four had positive, statistically significant correlations between rise-time and amplitude. The non-normality in significant numbers of cells may reflect heterogeneity between the synapses on a

given cell, but in cells where both amplitude and rise-time satisfied the normality test, the correlation was still evident.

We then explored the correlation between rise-time and amplitude in each cell. Plots from single cells generally showed no apparent clustering of points and displayed variable degrees of correlation. Two examples are shown for neurons, one with a strong and significant correlation (Fig. 4 A 1, $P < 0.0001$) and one with a weak insignificant correlation (Fig. 4 A 2, $P = 0.097$). Of the 29 neurons, 18 had plots with positive slopes and significant correlations and only 3 had negative slopes. The mean slope of all 29 neuron plots was 0.0117 ± 0.0028 msec/pA, somewhat higher than the slope from pooled data of 0.0077 msec/pA (Fig. 2 A) and binned data of 0.0089 msec/pA (Fig. 2 C). Of the six cells in which both quantities satisfied the Kolmogorov–Smirnov test

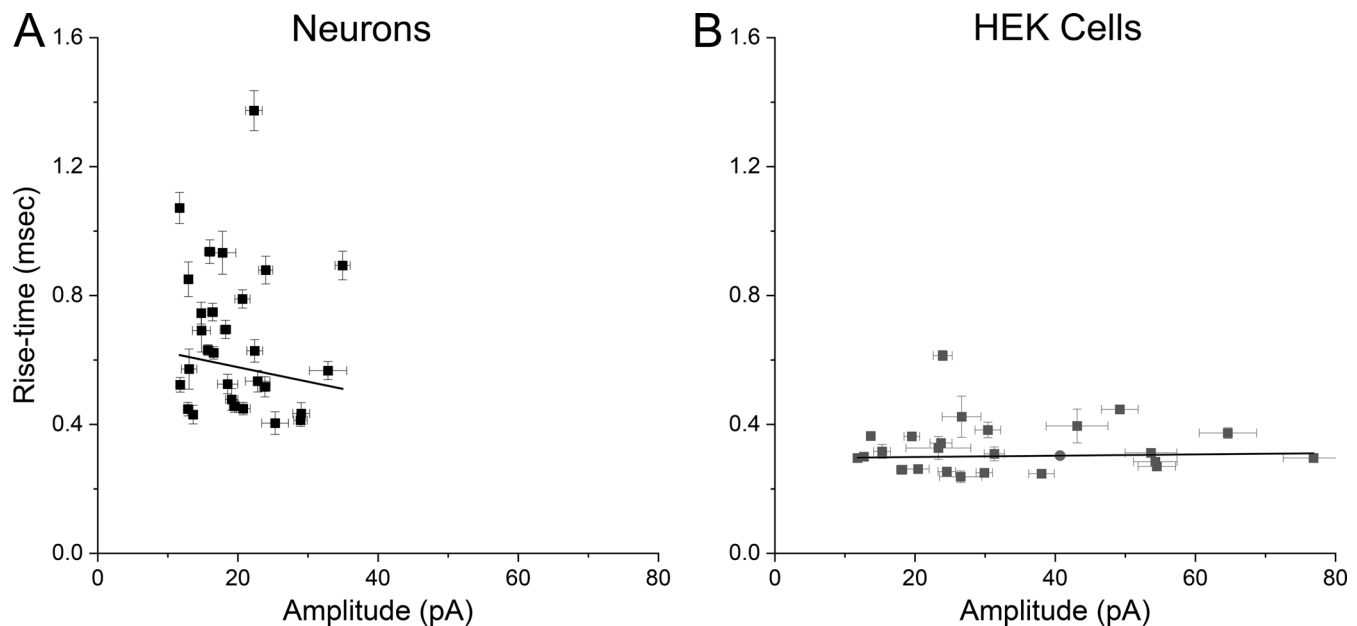


Figure 3. **Cell-mean plots of rise-time versus amplitude, with each point representing the means for an individual cell.** (A) Neurons: each point represents means of from 29 to 191 mEPSCs from an individual cell (mean = 109; slope = -0.0045 ± 0.0054 msec/pA). (B) HEK cells: each point represents a mean of from 16 to 174 mEPSCs from an individual cell (mean = 113; slope = 0.00020 ± 0.00094 msec/pA). Error bars are SE. Linear regression indicated no significant correlation in either plot (neurons $P = 0.413$; HEK cells $P = 0.833$). Note a few points have small error bars that are covered by the symbols.

for normality, the average slope was 0.0125 ± 0.0046 msec/pA, which is similar to the average just stated for all 29 cells. These six are less likely to have different populations of synapses. Turning to HEK cells, Fig. 4 B presents a plot with a positive slope that is not significantly different from zero ($P = 0.30$). 15 of the 24 HEK cells had significant correlations and 5 slopes were negative. The mean slope of the 24 HEK cell plots was 0.00123 ± 0.00088 msec/pA.

In summary, plots from single cells bear out the basic observations of pooled plots (Fig. 2, A and C), indicating a clear correlation between rise-time and amplitude in neurons. The slope was similar in neurons where both rise-time and amplitude appeared to be normally distributed. In HEK cells, the correlation is clearly weaker. The different results for statistical significance between single-cell and pooled data from HEK cells leave the question of the significance of the correlation unresolved, but it is clearly very weak, and the slope is far below that of neurons.

The comparison of Fig. 4, A 1 and A 2, shows that the slopes of amplitude versus rise-time plots varied widely between neurons. We found that neurons with longer mean mEPSC rise-times had steeper slopes (Fig. 4 C), and the two values were clearly correlated ($P < 0.0001$). In contrast, the plot from HEK cells indicated no significant correlation (Fig. 4 D, $P = 0.178$). Thus, while amplitude and rise-time do not co-vary between cells (Fig. 3), in neurons, the rise-times and slopes of amplitude-versus-rise-time plots do co-vary. In HEK cells they do not. This is another intriguing difference between neurons and HEK cells that will be investigated further below.

The foregoing analysis demonstrates that rise-times and amplitudes of mEPSCs can be correlated. Correlations of this form have received some prior attention and interpretations vary. In the neuromuscular junction, Negrete et al. (1972)

observed a correlation and suggested that it could reflect receptor saturation near the release site and diffusion of excess neurotransmitters to distal receptors. This form of correlation has also been interpreted in terms of the synchronous fusion of two vesicles, releasing more neurotransmitters to produce a larger amplitude mEPSC; jitter between the two release events then extends the rise-time (Raghavachari and Lisman, 2004). This interpretation requires that mEPSCs can be resolved into two or more populations. In our data, the scatter plots of pooled data (Fig. 2, A and B) show no indication of two clusters, and cells with normally distributed amplitudes and rise times also showed correlations. Finally, a mechanism not previously considered involves the dynamics of release through a fusion pore. For transmitter efflux through a static pore, it can be shown that flux will decay exponentially with a time constant proportional to vesicle volume (Almers et al., 1991). A larger vesicle will release more neurotransmitter and produce a larger amplitude mEPSC. Furthermore, with the same-sized fusion pore, a larger vesicle will take more time to lose its content than a smaller vesicle, and this will extend the rise-time. Fusion pore flux thus offers a potential explanation for the correlation between mEPSC amplitude and rise-time.

Other mechanisms that govern mEPSC shape predict uncorrelated or negatively correlated amplitudes and rise-times. An increase in cleft concentration will increase the amplitude and rate of receptor activation roughly in parallel, so amplitude will increase without a change in rise-time. In the calyx of Held, it was shown that increasing vesicular glutamate concentration increases amplitude and reduces rise-time, thus ruling out variations in intravesicular concentration as the mechanism (Guo et al., 2015). A higher receptor density will also increase

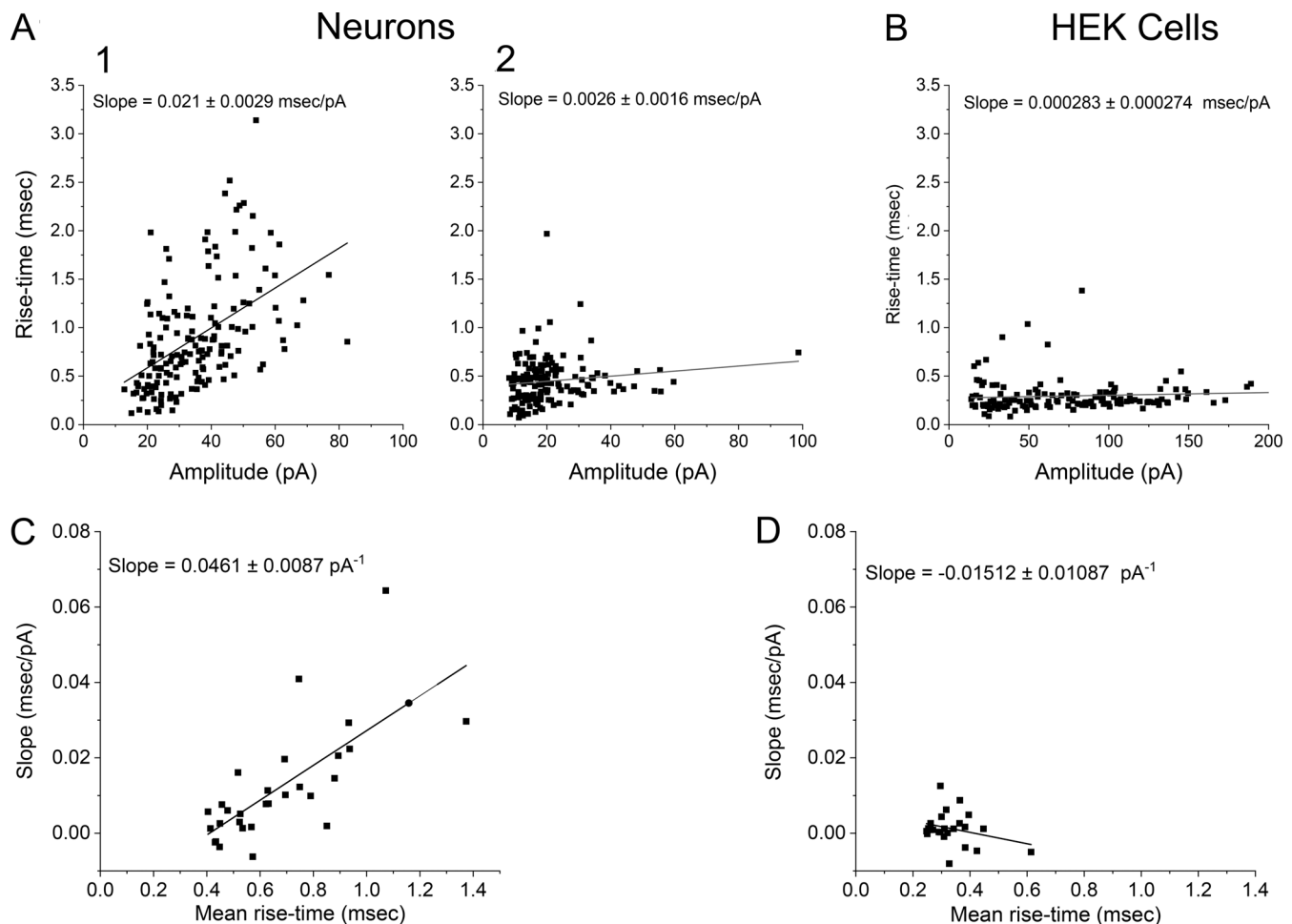


Figure 4. **Cell variations in rise-time-versus-amplitude plots.** (A 1) Rise-time-versus-amplitude plot of 160 mEPSCs recorded from one neuron. Linear regression indicated a highly significant correlation ($P < 0.0001$). (A 2) Plot of 150 mEPSCs recorded from another neuron. Linear regression indicated no significant correlation ($P = 0.097$). (B) Plot of 141 mEPSCs recorded from one HEK cell, with no significant correlation ($P = 0.30$). (C) Slopes from single-cell plots such as A 1 and A 2 were plotted versus the mean rise-time of the respective neuron. Linear regression indicated a highly significant correlation ($P < 0.0001$). (D) Slope versus mean rise-time plot for the 24 HEK cells showed no significant correlation ($P = 0.178$).

amplitude without changing the rise-time. Dendritic location is another well-known source of variation, but increasing the electrotonic distance of a synapse will reduce the amplitude and prolong the rise-time in parallel. This will lead to a relation opposite to that observed here. Dendritic filtering was in fact proposed previously to explain the slower rise-times of mEPSCs in neurons versus HEK cells (Chiang et al., 2021), but this interpretation is no longer tenable in light of the positive correlations revealed here in neurons (Fig. 2, A and C; and Fig. 4 A 1). Variations in registration between a presynaptic release site and postsynaptic receptors, such that exocytosis occurs at various distances from receptor clusters, will reduce amplitude and slow the rise-time, again producing the opposite relation. Plots in Figs. 2 and 4 thus present a challenge to interpretation and offer an opportunity to explore the mechanisms that shape mEPSCs in central synapses.

Simulations

To gain insight into how different properties of synapses can influence the shapes of mEPSCs and explore the variations and correlations just presented (Figs. 2, 3, and 4), we developed

models for synaptic receptor activation by transmitter released from a single vesicle. We first considered the relevant time scales to help choose factors to focus on. We developed a reduced model of fusion pore flux and receptor kinetics to explore the impact of these factors independent of the structural aspects of a synapse. Finally, we extended this model to include radial diffusion of the transmitter within the synaptic cleft.

Time scales

To guide model development, a few basic time scales were estimated. For diffusion in three dimensions, the root-mean-square displacement is $\sqrt{r^2} = \sqrt{6Dt}$ for time t . With a diffusion constant $D = 0.33 \mu\text{m}^2/\text{msec}$ (Nielsen et al., 2004), the time scale for diffusion across a synaptic cleft (thickness 20 nm) is very brief, 0.20 μsec . For diffusion radially within the plane of the cleft, we used the 2-dimensional result, $\sqrt{r^2} = \sqrt{4Dt}$. For a receptor nanodomain with a 50-nm radius (Biederer et al., 2017) the time is still quite short, 1.9 μsec . When the transmitter diffuses from a release site to receptors 100 nm away (Li et al., 2021), the timescale is 7.6 μsec . Since rise-times are on the order

of a few tenths of a millisecond (Table 1), these microsecond times are all much too short to be relevant to the shape of a mEPSC. Now consider spread from the release site to the perimeter of a synapse. For distances of 0.2, 0.5, and 1 μm from the center to the perimeter, we have time scales of 0.030, 0.19, and 0.75 msec, respectively. Thus, diffusion can only have an impact within the cleft of a very large synapse.

Another time scale to consider is for receptor activation. With a binding on-rate of $8 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$ (Krampfl et al., 2002) and a cleft concentration of 1 mM (Clements, 1996), the time scale is 0.125 msec. Based on the escape times just calculated, with a diameter of 0.2 μm , the glutamate will remain in the synapse for only 0.03 msec. Thus, 76% of the released neurotransmitter molecules will escape without binding to a receptor. For larger synapses, more neurotransmitters can bind before escaping. This ignores the fact that receptor activation requires multiple-site occupancy. So typical synapses will lose most of the neurotransmitter from a vesicle and see mostly unproductive single-site binding. The competition between binding and escape is consistent with the result that increasing the viscosity in the cleft increases mEPSC amplitude (Nielsen et al., 2004). Higher viscosity reduces the diffusion constant and increases the time neurotransmitter remains in the cleft where it can bind and activate receptors. The simulations presented below confirm this prediction.

Receptor modeling

We treat receptor binding and channel gating with a kinetic model for the AMPA-type glutamate receptor GluA2 expressed in HEK cells (Krampfl et al., 2002) (Fig. 5 A). This is the subunit we expressed in HEK cells (Chiang et al., 2021) and it is the most abundant AMPA-type receptor subunit in excitatory neurons (Wright and Vissel, 2012). The receptor state probabilities of Fig. 5 A ([R], [AR], [A₂R], [A₂O], [AD], [A₂D]) represent receptors with channels that are closed, R; open, O; and desensitized, D; and with zero, one, or two glutamate molecules; and A, bound. State occupancies change according to the following six coupled differential equations:

$$\frac{d[R]}{dt} = k_{-1}[AR] - k_1[A][R] \quad (1A)$$

$$\begin{aligned} \frac{d[AR]}{dt} = & k_1[A][R] + k_{-2}[A_2R] + d_{-2}[AD] - k_{-1}[AR] - k_2[A][AR] \\ & - d_2[AR] \end{aligned} \quad (1B)$$

$$\begin{aligned} \frac{d[A_2R]}{dt} = & k_2[A][AR] + d_{-1}[A_2D] + \alpha[A_2O] - k_{-2}[A_2R] - d_1[A_2R] \\ & - \beta[A_2R] \end{aligned} \quad (1C)$$

$$\frac{d[AD]}{dt} = d_2[AR] + k_{-3}[A_2D] - d_{-2}[AD] - k_3[A][AD] \quad (1D)$$

$$\frac{d[A_2D]}{dt} = k_3[A][AD] + d_1[A_2R] - k_{-3}[A_2D] - d_{-1}[A_2D] \quad (1E)$$

and

$$\frac{d[A_2O]}{dt} = \beta[A_2R] - \alpha[A_2O]. \quad (1F)$$

The values of the rate constants in these equations are given in the legend of Fig. 5 A. Neurotransmitter concentration, [A],

changes according to the following equation (Chang et al., 2017; Chiang et al., 2018):

$$[A(t)] = \frac{N_0}{4\pi D\tau} \int_0^{t-\epsilon} \frac{e^{-s/\tau}}{t-s} ds. \quad (2)$$

Note that this expression assumes excess transmitter and neglects changes in [A] due to receptor binding. Eq. 2 is the convolution integral of the fusion pore flux (which decays exponentially with time, as $e^{-s/\tau}$), with the solution of the diffusion equation in radial coordinates. The spatial coordinate is set to $r = 0$ to represent the concentration directly under the release site. t is the time after fusion pore opening. ϵ is the time to diffuse over a distance equal to the thickness of the synaptic cleft, 0.2 μm (calculated above), and its incorporation removes the singularity at $s = t$. The results are insensitive to the precise value of ϵ . Because Eq. 2 represents [A] at the site of vesicle fusion, where $r = 0$, radial diffusion is not directly treated, but does come into play as it determines the speed with which the released transmitter escapes from the synapse.

The exponential term $e^{-s/\tau}$ in Eq. 2 arises from the hypothesis of a static fusion pore with a flux proportional to intravesicular concentration (Almers et al., 1991). Content and flux decay exponentially. τ is given as a product of the fusion pore permeability times the vesicle volume; the permeability does not depend on vesicle size. The vesicle volume is proportional to N_0 , the initial number of molecules contained in the vesicle. In endocrine vesicles, direct measurements of vesicle membrane area and content indicate a scaling to maintain a constant concentration (Gong et al., 2003). At the *Drosophila* neuromuscular junction, a scaling between vesicle volume and quantal size also suggests a fixed intravesicular glutamate concentration (Karunanithi et al., 2002). Thus, N_0 serves as a surrogate for size. We represent τ by αN_0 , where α includes the pore permeability and intravesicular concentration. We will express this dependence as

$$\tau = \alpha N_0 / 6,000. \quad (3)$$

This serves as a convenient representation, where α is the time constant for a vesicle containing 6,000 molecules (the choice of 6,000 will be discussed shortly below).

All receptor state occupancy probabilities in Eqs. 1 A, B, C, D, E, and F vary between 0 and 1; the initial value of [R] at $t = 0$ is 1 and all the others are zero; the kinetic equations obey detailed balance and constrain the sum of the 6 receptor state probabilities to 1. This model enables us to focus on the dynamic interaction between fusion pore flux and receptor kinetics. By taking the synapse's geometric center ($r = 0$) and ignoring spatial variations in concentration and receptor activation, there are no gradients. The full system of equations was solved using Mathcad to obtain the open channel probability, [A₂O], versus t .

In addition to the rate constants for GluA2 (Krampfl et al., 2002 and Fig. 5 A), we also need values for N_0 , the number of neurotransmitter molecules in a vesicle, α of Eq. 3 to set the fusion pore time constant, and D , the coefficient of diffusion of neurotransmitter in the cleft. Estimates of N_0 in central glutamatergic synapses vary but a recent direct amperometric measurement obtained 8,000 for isolated vesicles and 5,000 for

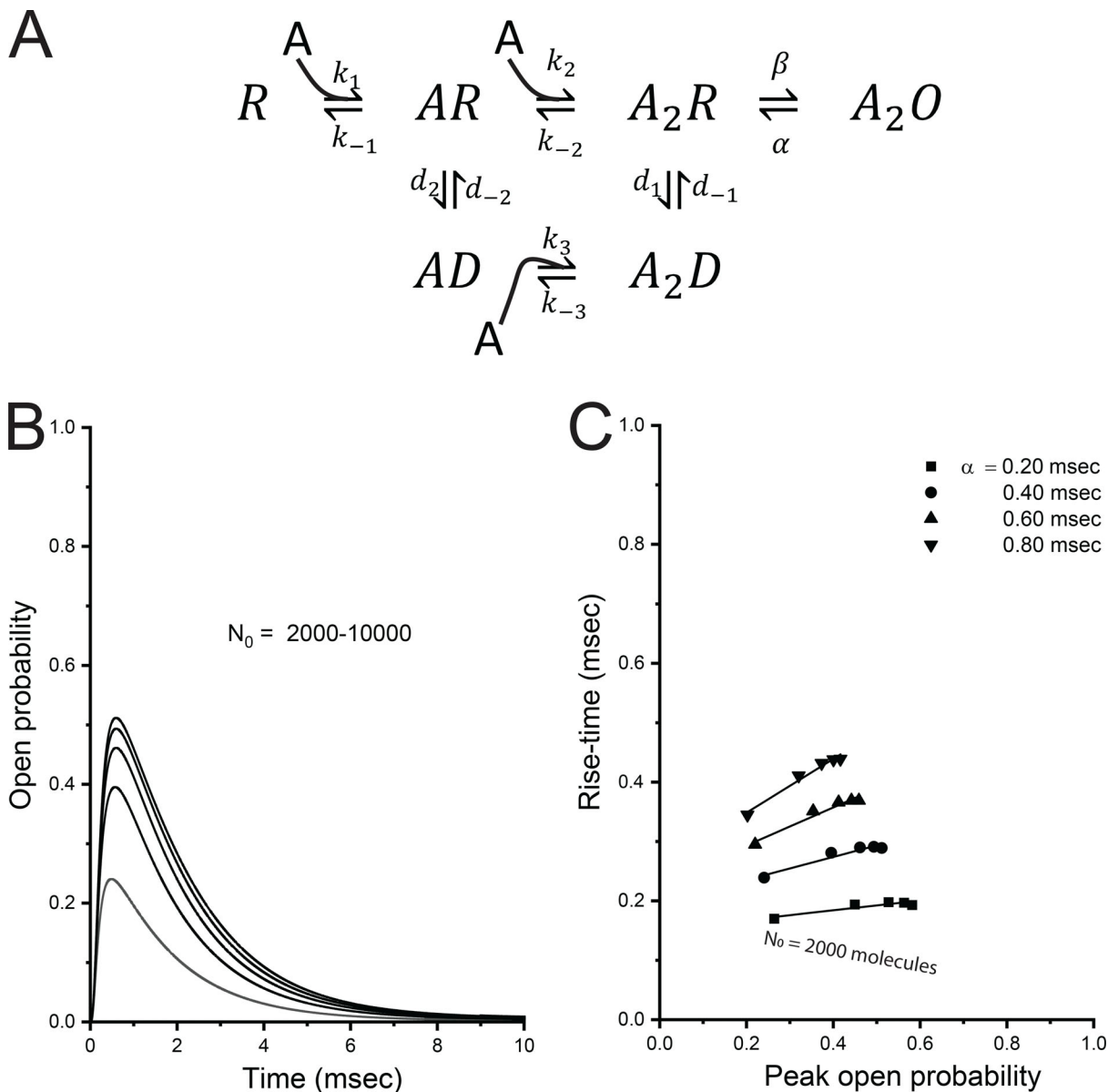


Figure 5. **Fusion pore flux and receptor dynamics.** (A) Model for receptor activation. Neurotransmitter molecules, A, bind to a receptor, R, which can interconvert to an open channel, O, or a desensitized state, D. Rate constants for the interconversions are indicated for the forward and reverse steps. Values were: $k_1 = 0.008 \mu\text{M}^{-1}\text{msec}^{-1}$; $k_2 = k_3 = 0.004 \mu\text{M}^{-1}\text{msec}^{-1}$; $k_{-1} = 2.0 \text{ msec}^{-1}$; $k_{-2} = 4 \text{ msec}^{-1}$; $k_{-3} = 0.0622 \text{ msec}^{-1}$; $d_1 = 4.5 \text{ msec}^{-1}$; $d_{-1} = 0.007 \text{ msec}^{-1}$; $d_2 = 0.6 \text{ msec}^{-1}$; $d_{-2} = 0.06 \text{ msec}^{-1}$; $\alpha = 2.4 \text{ msec}^{-1}$; $\beta = 20 \text{ msec}^{-1}$ (model and rate constants from Krampfl et al., 2002). (B) Simulations of open probability versus time for $\alpha = 0.4 \text{ msec}$ (see Eq. 3), $D = 0.3 \mu\text{m}^2/\text{msec}$, and the indicated values of N_0 . (C) Plots of rise-time versus peak open probability. Each plot includes five points for $N_0 = 2,000$ – $10,000$, and the indicated values for α .

monophasic spontaneous release events from neurons (Wang et al., 2019). We will explore the range $N_0 = 2,000$ – $10,000$ and take $N_0 = 6,000$ when it is not varied. Flux through the fusion pore must be fast enough to enable the glutamate concentration to rise to the millimolar range (Clements, 1996). $\alpha \sim 0.5 \text{ msec}$ accomplishes that with $N_0 = 6,000$, we will explore α ranging from 0.2 to 0.80 msec. The diffusion constant, D, has been estimated to be two to threefold slower in the synaptic cleft than in the free solution (Nielsen et al., 2004; Zheng et al., 2017). We will take $D = 0.3 \mu\text{m}^2/\text{msec}$, a value used in previous modeling studies in the neuromuscular junction (Wathey et al., 1979) and calyx of Held (Budisantoso et al., 2013).

Fig. 5 B presents simulations of open probability ($[A_2O]$) versus time for $N_0 = 2,000$ – $10,000$. As expected, the peak open probability increases as N_0 increases. The peak also shifts to the right as the rise-time increases. Rise-times (10–90% for all simulations) are plotted versus peak open probability in Fig. 5 C. The positive slopes qualitatively agree with experiments in neurons (Fig. 2, A and C; and Fig. 4 A 1). A range of α values was tested as indicated in the legend. The trend is clear that for longer α (less permeable fusion pores) both the rise-times and the slopes of the plots increase in parallel. Thus, the qualitative nature of the correlations can be recapitulated without considering structural detail or radial diffusion. The fractional changes

in rise time with large α values are comparable with those observed in neurons, and the slopes will be compared quantitatively in simulations of the current with a more detailed model below. Fig. 5 C reveals the important role of fusion pores in the correlation between mEPSC rise-time and amplitude. For large fusion pores (low α), even the largest vesicles lose transmitter so rapidly that receptor activation limits the rise rate. For smaller fusion pores (large α), the extended time of transmitter efflux becomes rate-limiting. The plots are shifted upward with smaller fusion pores and the slopes increase in parallel. This recapitulates the experimental behavior in neurons of a positive correlation between rise-time and slope (Fig. 4 C). Finally, we also note that the range of rise-times in Fig. 5 C fell below the experimental value for neurons (Table 1). The model with radial gradients will address this discrepancy.

Incorporating radial gradients

To gain insight into the role of synapse structure and receptor density, we extended the above model to incorporate radial diffusion within the synaptic cleft and spatial gradients. Our approach roughly follows previous mEPSC models in the neuromuscular junction (Wathey et al., 1979; Land et al., 1980), and Fig. 6 A illustrates the basic features. Neurotransmitter exits a vesicle through a fusion pore with time constant τ and then diffuses radially outward within the cleft as it binds receptors distributed uniformly within a circular patch of receptor-rich postsynaptic membrane centered at the release site and extending to a perimeter at radius r_p . The density of receptors in this circle is R_0 in molecules/ μm^2 . Neurotransmitter uptake is not included as the blockade of the glutamate transporter has been shown to leave mEPSCs unchanged (Mennerick and Zorumski, 1995).

The model includes the same equations for receptor activation (Eqs. 1, A, B, C, D, E, and F), but all of these receptor states now depend on r , the distance from the release site, as well as t . Instead of Eq. 2 for the derivative of $[A]$, we now use the following expression:

$$\frac{\partial[A]}{\partial t} = D \left(\frac{\partial^2[A]}{\partial r^2} + \frac{1}{r} \frac{\partial[A]}{\partial r} \right) + \frac{[A]_0}{\tau} e^{-\frac{r^2}{2\sigma^2}} + k_{-1}[AR] + k_{-2}[A_2R] + k_{-3}[A_2D] - k_1[A][R] - k_2[A][AR] - k_3[A][AD]. \quad (4)$$

This describes the movement of A within the cleft; $[A]$ depends on r and t . The spatial variations in $[A]$ drive the spatial variations in receptor state probabilities. The first term on the right side is the spatial part of the diffusion equation in radial coordinates. The second represents the exponential loss of transmitter from the vesicle through the fusion pore (with τ given by Eq. 3). This term serves as a source distributed as a 2-dimensional Gaussian in the plane of the cleft centered at $r = 0$. The width of this Gaussian, σ , is taken as the synaptic cleft width of 20 nm. This circumvents the computational difficulty of expressing the initial concentration as a delta function. The second line of Eq. 4 expresses changes in $[A]$ due to receptor binding and unbinding, and the degree of receptor saturation can vary. The receptor states were initialized as $[R] = R_0$ for the unliganded

state and 0 for all the others. The system of equations conserves the sum of the six receptor states at each location to maintain a density of R_0 .

Values for N_0 , α , D , and GluA2 rate constants were discussed above. This extended model now also requires values for R_0 and r_p . Studies suggest that AMPA-type receptors have post-synaptic densities roughly in the range of 1,000–3,000 μm^{-2} (Franks et al., 2003; Harris et al., 2012). We will test the range of 1,000–5,000 μm^{-2} and assume a uniform distribution because the time scales calculated above suggest ~ 50 nm receptor clusters (Sheng and Hoogenraad, 2007; Biederer et al., 2017) are unlikely to be relevant. For r_p , we note that postsynaptic density dimensions fall in the range of 0.2–0.8 μm (Sheng and Hoogenraad, 2007), and the mean area has been estimated as 0.04 μm^2 (Schikorski and Stevens, 1997). We will take $r_p = 0.2$ μm in most simulations and explore the range 0.1–0.5 μm .

This system of equations was solved with MATLAB. Temporal and spatial graining were varied to test and ensure accuracy. A boundary at $r = 5$ μm was set as either absorbing or reflecting and had no impact on the results because 5 μm was well beyond the region where concentrations were significant. Runs of the code generated transmitter and receptor state occupancy as a function of r and t . The transmitter number per unit area was converted to concentration by dividing by cleft thickness and Avogadro's number. Open channel occupancy, $[A_2O]$, was summed over the area (from $r = 0$ to r_p) to get the total number of activated channels versus time. This was converted to current by multiplying by -0.5 pA, which is the product of the holding potential -65 mV and single channel conductance of 7.6 pS (Krampfl et al., 2002). These currents versus time are presented as simulated mEPSCs.

To explore how synapse properties shape mEPSCs, simulations were performed with varying values of N_0 (Fig. 6 B), R_0 (Fig. 6 C), r_p (Fig. 6 D), α (Fig. 6 E), and D (Fig. 6 F). N_0 variations produced a wide range of amplitudes, with smaller increments at high N_0 due to receptor saturation (Fig. 6 B). N_0 (and thus vesicle size) is likely to be the main source of mEPSC amplitude fluctuations. High R_0 and r_p also saturated amplitude because 10,000 transmitter molecules could not activate more receptors, whether they were denser (Fig. 6 C) or available over a larger area (Fig. 6 D). Varying α predominantly impacted rise-time (Fig. 6 E). Varying D had a greater impact on amplitude (Fig. 6 F).

Fig. 6, B–F, all have insets with plots of rise-time versus amplitude. These plots bring out the strikingly different impacts of the parameters. Varying N_0 produced a positive slope (Fig. 6 B inset) as seen experimentally in neurons (Figs. 2 and 4) and with the more basic model (Fig. 5 C). Varying R_0 produced a flat plot with essentially zero slope (Fig. 6 C inset). Fig. 6 D inset shows that varying r_p produced a steep plot similar to that with varying N_0 . Varying α produced a large negative slope (Fig. 6 E inset). Varying D produced a very small positive slope (Fig. 6 F inset); the large change in amplitude and small change in rise-time are consistent with the impact of experimentally manipulating D (Nielsen et al., 2004). The impacts of R_0 and α are nearly orthogonal: varying R_0 produces large changes in amplitude and essentially no change in rise-time while varying α does nearly the opposite. This orthogonality is significant, and the discussion

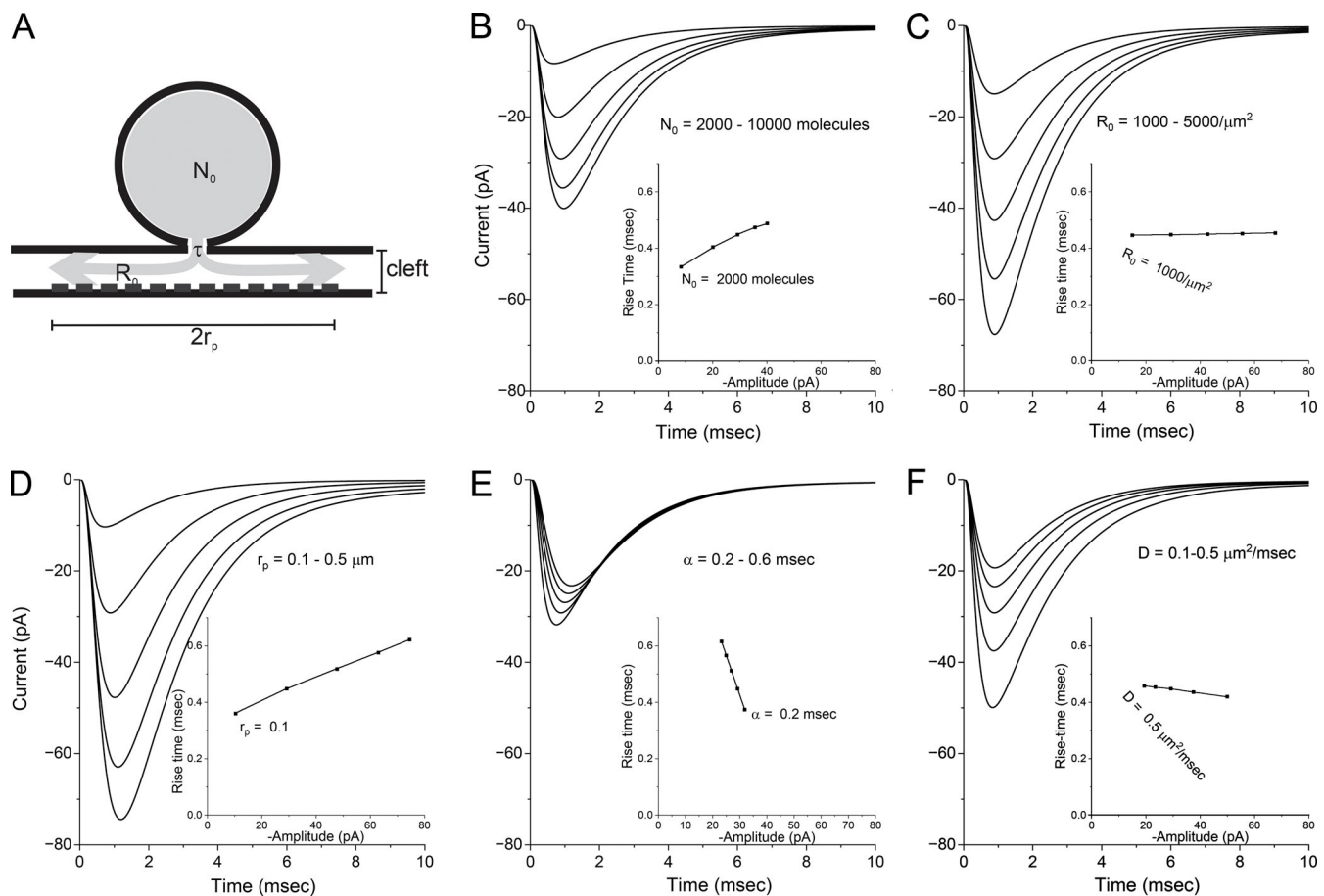


Figure 6. Fusion pore flux, receptor dynamics, and diffusion. (A) A model for neurotransmitter flux and radial diffusion. Receptor activation is represented by Eqs. 1, A, B, C, D, E, and F, according to the kinetic model for GluA2 (Fig. 5 A). A vesicle initially contains N_0 molecules of neurotransmitter that escape through a fusion pore with a time constant for flux of τ (Eq. 3). After passing through the fusion pore, neurotransmitter diffuses radially as indicated by the grey arrows. Neurotransmitter activates receptors in the postsynaptic membrane with a density R_0 . r_p is the radius of a circular receptor-rich region of postsynaptic membrane and thus represents the size of the synapse. (B) Simulated mEPSCs with variation in N_0 . (C) Variation in R_0 . (D) Variation in r_p . (E) Variation in α (and thus τ). (F) Variation in D . Insets in B–F show plots of rise-time versus amplitude for the varied parameter. When not varied simulations were performed with $N_0 = 6,000$ molecules, $R_0 = 2,000 \mu\text{m}^{-2}$, $r_p = 0.2 \mu\text{m}$, $\alpha = 0.30$ msec, and $D = 0.3 \mu\text{m}^2/\text{msec}$. B–F all include one simulation with the same parameters (peak amplitude -29.2 pA).

below will explore its relevance to variations in neurons versus HEK cells (Fig. 3).

Rise-time amplitude correlations

To explore the basis for the observation that neurons with longer mean rise-times have steeper rise-time versus amplitude plots (Fig. 4 C), we varied parameters in an effort to replicate this behavior. We varied $R_0 = 1,000$ – $4,000 \mu\text{m}^{-2}$ for two different values of r_p (Fig. 7, A 1 and A 2). The points at $N_0 = 6,000$ are highlighted with circles to illustrate how a plot of amplitude versus rise-time would be influenced. The nearly horizontal rows formed by these points resemble the horizontal plot in Fig. 6 C. The highlighted rise-times form the x-axis for plots of slope versus rise-time (Fig. 7 A 3). These plots look nothing like the experimental plot in Fig. 4 C, indicating that variations in R_0 between cells cannot explain the correlation between slope and rise-time. The finding that amplitude can vary so widely without variations in rise-time recapitulates the behavior of these quantities in HEK cells (Fig. 3 B).

Varying r_p generated plots with positive correlations between rise-time and amplitude with both $R_0 = 1,000 \mu\text{m}^{-2}$ (Fig. 7 B 1) and $4,000 \mu\text{m}^{-2}$ (Fig. 7 B 2), but the slopes decreased with increasing rise-time (Fig. 7 B 3). Varying D (Fig. 7, C 1 and C 2) generated very steep plots with positive slopes (Fig. 7 C 3), but the range of rise-times was much narrower than that observed experimentally. Thus, variations in R_0 , r_p , and D failed to account for the observed behavior (Fig. 4 C).

Turning to α , we see that for both $R_0 = 1,000$ (Fig. 7 D 1) and $4,000$ (Fig. 7 D 2), increasing α increased both the rise-time and the slope of plots, as seen with the reduced model (Fig. 5 C). Using the points with $N_0 = 6,000$ (circled in Fig. 7, D 1 and D 2) to generate a plot of slope versus rise-time generated plots resembling that from neurons. The slopes with $R_0 = 1,000$ and $4,000/\mu\text{m}^2$ of 0.018 and 0.068 pA^{-1} , respectively, bracket the slope from the experimental plot in Fig. 4 C of 0.046 pA^{-1} . This indicates that $R_0 \sim 3,000/\mu\text{m}^2$ will give a slope in agreement with the observed value, and this density is consistent with experiments (Franks et al., 2003; Harris et al., 2012).

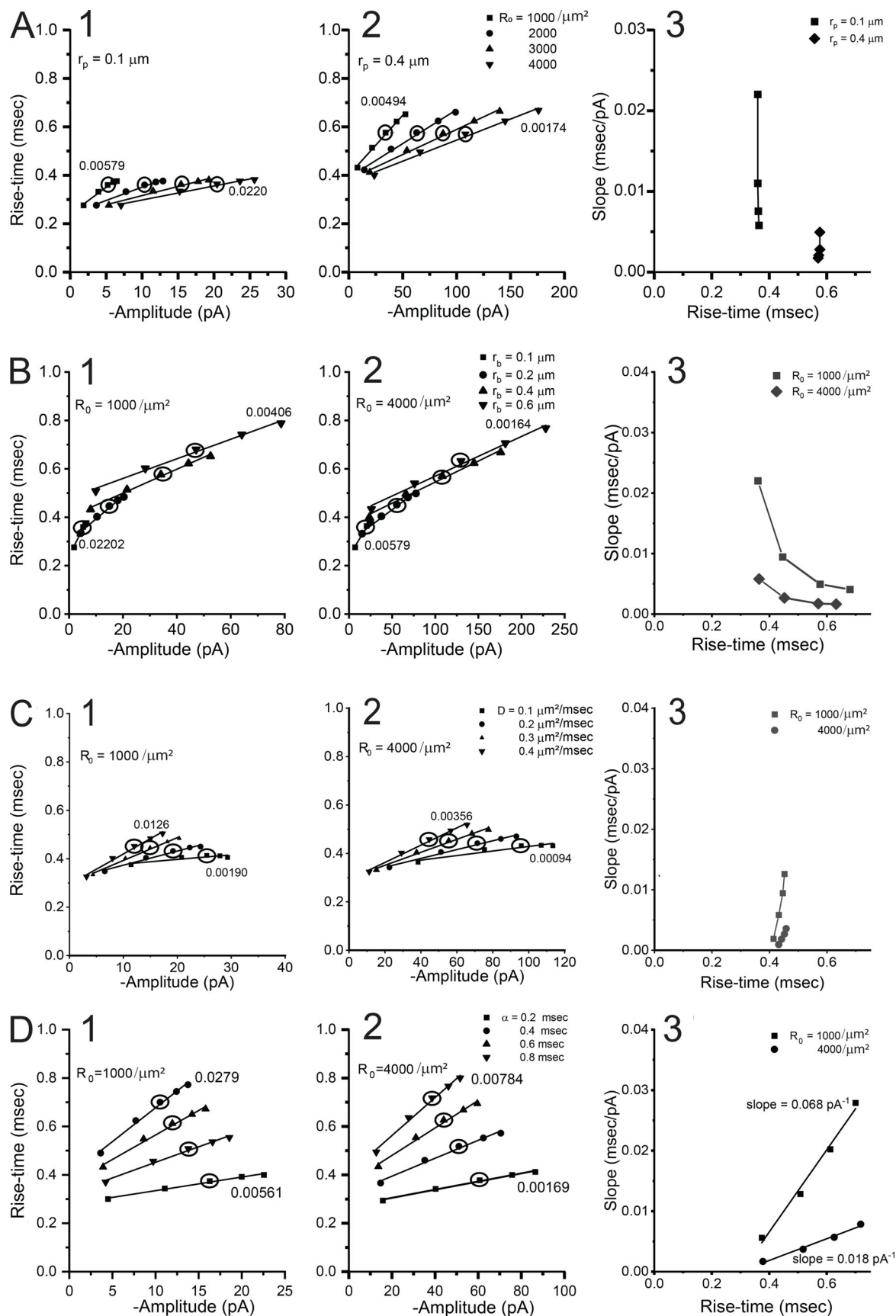


Figure 7. **Plots of rise-time versus amplitude from simulations with $N_0 = 2,000\text{--}10,000$.** (A 1 and 2) Plots for $R_0 = 1,000\text{--}4,000 \mu\text{m}^{-2}$ with $r_p = 0.1 \mu\text{m}$ (A 1) and $0.4 \mu\text{m}$ (A 2), and $\alpha = 0.3 \text{ msec}$. Circles highlight points for $N_0 = 6,000$ throughout the figure. (A 3) The slopes of the plots in A 1 and A 2 are plotted

versus the rise-time with $N_0 = 6,000$ (A 3). **(B 1–3)** Plots with $r_p = 0.1, 0.2, 0.4$, and $0.6 \mu\text{m}$ for $R_0 = 1,000 \mu\text{m}^{-2}$ (B 1) and $4,000 \mu\text{m}^{-2}$ (B 2 and B 3). **(C 1–3)** Plots with D from 0.1 to $0.4 \mu\text{m}^2/\text{msec}$ for $R_0 = 1,000 \mu\text{m}^{-2}$ (C 1) and $4,000 \mu\text{m}^{-2}$ (C 2). Slopes plotted versus rise-times with $N_0 = 6,000$ (C 3). **(D 1–3)** Plots with $\alpha = 0.2$ – 0.8 msec for $R_0 = 1,000 \mu\text{m}^{-2}$ (D 1) and $4,000 \mu\text{m}^{-2}$ (D 2). Slopes plotted versus rise-time with $N_0 = 6,000$ (D 3). Highest and lowest slope values (units of msec/pA) are presented for comparison with experimental values (Figs. 2 and 4). Only variations of α in D 1 and D 2 generated plots with slopes that varied as observed in experiments; the correlations between slope and rise-time in D 3 were similar to that observed in neurons (Fig. 4 C).

mEPSCs in HEK cells

Based on these insights into the impacts of parameter variations, we sought to recapitulate mEPSCs in HEK cells. Given the apparent orthogonality of R_0 and α in their influences on amplitude and rise-time (Fig. 6, B and E), we varied those two parameters to recover the values for HEK cells in Table 1, while maintaining $N_0 = 6,000$, $r_p = 0.2 \mu\text{m}$, and $D = 0.3 \mu\text{m}^2/\text{msec}$. With $R_0 = 1,970$ and $\alpha = 0.15$ msec, a simulated mEPSC (Fig. 8 A) had an amplitude of -32.76 pA and a rise-time of 0.334 msec. The slope of the plot of rise-time versus amplitude (Fig. 8 B) of 0.00205 msec/pA is somewhat higher than the slope in Fig. 2 D as well as the cell-mean slope, but within the margin of error.

mEPSCs in neurons

The mean rise-time in neurons of 0.663 msec is twice that of 0.332 msec in HEK cells (Table 1). We had originally attributed the longer rise-times in neurons to dendritic attenuation (Chiang et al., 2021), but the positive slope in the plots of rise-time versus amplitude rules this out. To search for a better explanation, we will test two possibilities. We first followed the strategy illustrated above with HEK cells and varied R_0 to fit the

amplitude and α to fit the rise-time. The simulated mEPSC with $R_0 = 1,800 \mu\text{m}^{-2}$ and $\alpha = 0.70$ msec (Fig. 9 A, GluA2) had an amplitude of -19.7 pA and a rise-time of 0.662 msec, in agreement with the values for neurons in Table 1. This value of α is somewhat larger than the value for τ_{decay} of 0.4 msec for glutamate clearance from synapses in cultured hippocampal neurons (Diamond and Jahr, 1997). Simulating mEPSCs for $N_0 = 2,000$ – $10,000$ generated a plot of rise-time versus amplitude with a slope of 0.0137 msec/pA (Fig. 8 B), which is somewhat higher than the slope of the pooled experimental plot for neurons (Fig. 2 C), and quite close to the cell-mean slope. Thus, values of R_0 and α adjusted to the neuronal mEPSC amplitude and rise-time recapitulated the relation between rise-time and amplitude with no further adjustments of parameters.

Another potential factor in comparing rise-times between neurons and HEK cells is their glutamate receptors. The HEK cells expressed only GluA2 receptors, and our kinetic model was based on experiments in HEK cells expressing this protein (Krampfl et al., 2002). Thus, the receptor model employed in this study is especially well suited for the HEK cell data. By contrast, neurons express GluA2 along with other subunits and

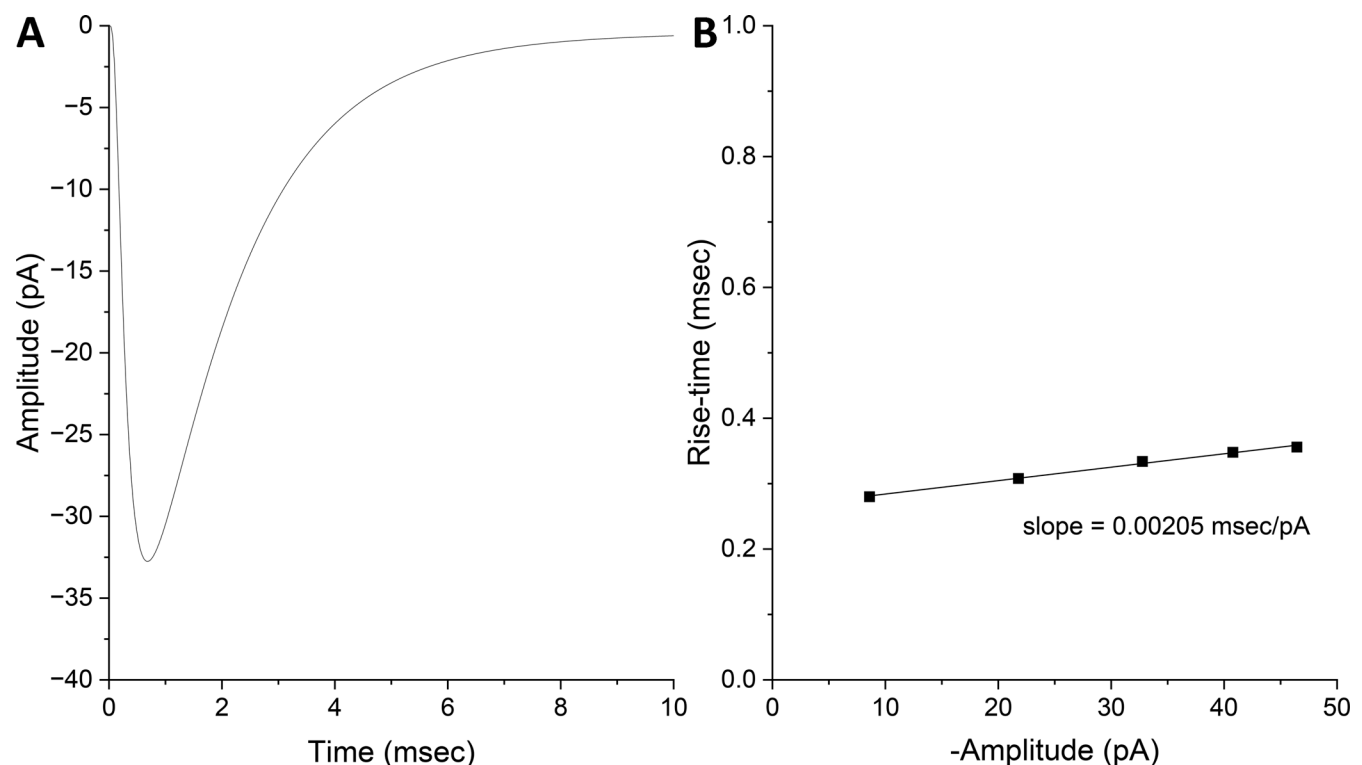


Figure 8. **Recapitulation of HEK cell mEPSCs.** (A) An mEPSC was simulated with $N_0 = 6,000$, $r_p = 0.2 \mu\text{m}$, $R_0 = 1,970$ receptors/ μm^2 , $\alpha = 0.15$ msec, and $D = 0.3 \mu\text{m}^2/\text{msec}$. The peak amplitude was -32.76 pA and the rise-time was 0.334 msec, in agreement with the values in Table 1. (B) With N_0 varied from $2,000$ to $10,000$, the rise-time was plotted versus amplitude and fitted to a line with a slope of 0.00205 msec/pA.

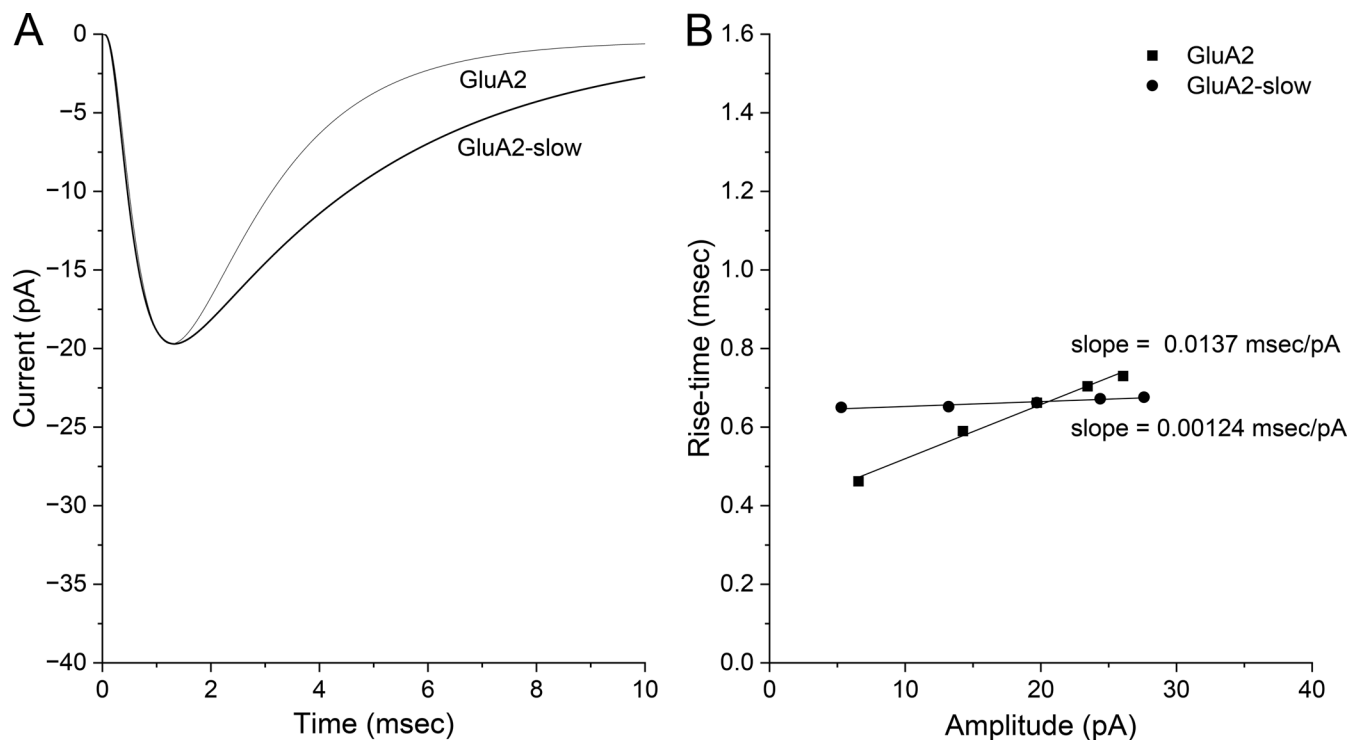


Figure 9. **Recapitulation of mEPSCs in neurons.** (A) With GluA2 parameters from the legend of Fig. 5, $N_0 = 6,000$, $r_p = 0.2 \mu\text{m}$, and $D = 0.3 \mu\text{m}^2/\text{msec}$, R_0 was adjusted to $1,800 \mu\text{m}^{-2}$ and α to 0.70 msec . The mEPSC (labeled GluA2) had a peak amplitude of -19.70 pA and rise-time of 0.662 msec , in agreement with the experimental values of neurons (Table 1). The trace labeled GluA2-slow kept the value of $\alpha = 0.15$ from the HEK cell simulation (Fig. 8) and modified GluA2 parameters by multiplying β , k_{-2} , and d_1 by 0.0485 . N_0 , r_p , and D were kept the same and R_0 was adjusted to $2,704 \mu\text{m}^{-2}$. This simulated mEPSC had the same peak amplitude and rise-time and a slower decay. (B) Varying N_0 from $2,000$ to $10,000$ for GluA2 and GluA2-slow generated a series of mEPSCs. Peak amplitudes and rise-times are plotted and the slopes are indicated in the figure. The GluA2 slope was close to that for neurons (Fig. 2 C); the GluA2-slow slope was much lower and outside the margin of error.

form hetero-oligomers (Wright and Vissel, 2012). The subunit composition of AMPA receptors can influence mEPSC rise-times (Yang et al., 2011). Additional GluA subunits could account not only for the difference in rise-time but also the nearly twofold slower decays of mEPSCs in neurons (Chiang et al., 2021). The HEK cells used for these studies already express four different proteins (GluA2, neuroligin, PSD-95, and stargazin), and it would be impractical to add more. Furthermore, the complexity and heterogeneity of glutamate receptors in neurons make choosing additional subunits very difficult. Instead, we choose to simulate slower receptors by varying the rate constants of the model in Fig. 5 A, while retaining the value of $\alpha = 0.15 \text{ msec}$ from HEK cells (Fig. 8). This approach addresses the question of how well slower receptor kinetics can simulate neuronal mEPSCs. We first attempted to slow the mEPSC onset by reducing the binding rates, k_1 , k_2 , and k_3 in the kinetic model (Fig. 5 A). This only reduced the amplitude because slower binding primarily reduced the number of receptors activated in the narrow time window of neurotransmitter exposure. Varying other parameters indicated that mEPSC rise-times depend most strongly on the lifetime of state A_2R in Fig. 5 A, which is determined by the three exit rates from A_2R , namely β , k_{-1} , and d_1 . Reducing these three parameters by the same factor proved to be an effective way of slowing the mEPSC rise-time. Keeping α at 0.15 msec from HEK cells and using the same values of N_0 , r_p , and D , we

adjusted R_0 to $2,704 \mu\text{m}^{-2}$ and multiplied β , k_{-2} , and d_1 by 0.0485 . The resulting simulated mEPSC had a peak amplitude of -19.70 pA and a rise-time of 0.662 msec (Fig. 9 A, GluA2-slow), in agreement with the experimental values from neurons (Table 1). The simulated mEPSC for GluA2-slow nearly overlies that for GluA2 from onset through peak but decays more slowly. While GluA2-slow prolonged the rise-time to that seen in neurons, the slope of the rise-time versus amplitude plot was much lower (0.00124 msec/pA ; Fig. 9 B). This suggests that in contrast to slower fusion pore flux, slower receptor kinetics are less effective in reconciling the difference in rise-times between HEK cells and neurons.

Discussion

This study explored the mechanisms that shape quantal synaptic responses, focusing on a previously reported correlation between amplitude and rise-time (Negrete et al., 1972; Hartzell et al., 1975; Wathey et al., 1979; Wall and Usowicz, 1998; Magee and Cook, 2000; Barberis et al., 2004; Guo et al., 2015). We observed this correlation in mEPSCs from synapses between neurons in both pooled data from many neurons as well as single-cell plots. In synapses on HEK cells, the correlation was much weaker and its statistical significance depended on whether we looked at pooled or single-cell data. Furthermore,

we found that neurons with longer mean rise-times had steeper slopes in their rise-time versus amplitude plots. Although this higher-level correlation has received little prior attention, it can be seen in published data (Magee and Cook, 2000; Guo et al., 2015).

To investigate mechanisms underlying these correlations, we used models to simulate mEPSCs. A reduced model simulated flux through a fusion pore and activation of receptors (Fig. 5 A), and a more detailed model added radial neurotransmitter diffusion and spatial gradients in receptor activation (Fig. 6 A). Simulations suggested that vesicle size, through its surrogate N_0 , is the principal source of quantal variation within a cell. Amplitudes and rise-times vary between neurons and HEK cells. Greater variations in receptor density, R_0 , can account for the wider variation in amplitude between HEK cells (Fig. 3 B), while greater variations in fusion pores (α) can account for the wider variation in rise-time between neurons (Fig. 3 A). The orthogonal simulation results with R_0 (Fig. 6 C) versus α (Fig. 6 E) mirror the roughly orthogonal experimental results with cell-means in neurons (Fig. 3 A) versus HEK cells (Fig. 3 B).

The detailed model recapitulated both neuronal and HEK cell mEPSCs using reasonable parameter values. Our simulations robustly recapitulated the correlation between rise-time and amplitude, showing these quantities co-vary as N_0 varies (Fig. 5 C and Fig. 7 D). This finding offers an interpretation of the correlation in terms of the extended time for transmitter escape from larger vesicles. Furthermore, variations in the parameter representing fusion pore permeability, α , were also strikingly successful in recapitulating the higher-level correlation between rise-time and rise-time-versus-amplitude slope. The plot from simulations (Fig. 7 D 3) resembles the plot from experimental data (Fig. 4 C). The comparison between neurons and HEK cells also illustrates this higher-level correlation: the briefer rise-times in HEK cells fit with their weakly correlated plots of rise-time-versus-amplitude. Similar observations can be made in data from hippocampal pyramidal cells (Magee and Cook, 2000) and calyx of Held (Guo et al., 2015).

The positive correlation between rise-time and amplitude of simulated EPSCs depended critically on assuming that N_0 plays a role in the time course of neurotransmitter loss (Eq. 3). Extensive simulations without this dependence of τ on N_0 were only able to achieve positive slopes with unrealistically large values of r_p ($\sim 0.8 \mu\text{m}$) and low values of D ($\sim 0.1 \mu\text{m}^2/\text{msec}$). Even then the slopes fell well below those seen in experiments. With $r_p = 0.2 \mu\text{m}$, $R_0 = 2,000$, and $D = 0.3 \mu\text{m}^2/\text{msec}$, the slope of rise-term versus amplitude plot was slightly negative. Thus, diffusion extending the rise-time with large vesicles (Negrete et al., 1972) can account for only part of the correlation at best. By contrast, a model with no radial diffusion was qualitatively successful (Fig. 5). The recapitulation of this correlation by incorporating a vesicle size dependence of fusion pore flux into the model thus supports the concept of exponential loss of transmitter from a vesicle through a static pore (Almers et al., 1991). The exponential decay indicates that flux is proportional to concentration and that the fusion pore is not saturated by the neurotransmitter even at the high initial concentration. The fusion pore is stable for ~ 1 msec as the transmitter pours out, and it is the same size

for small and large vesicles. The picture that emerges is that small vesicles lose their transmitter rapidly, leaving receptor activation as the rate-limiting step; it takes more time for a large vesicle to release transmitter, so receptor activation then tracks release. The higher-level correlation with rise-time emerges because larger fusion pores that make rise-times fast will leave receptor activation as rate limiting even for large vesicles. Smaller fusion pores that make rise-times slow also allow vesicle size to have a greater impact on rise-time.

This study suggests that synapses formed by neurons with other neurons have smaller and more variable fusion pores compared with synapses formed by neurons with HEK cells. Although our efforts to adjust receptor rate constants (Fig. 9, GluR-slow) cannot entirely rule out a contribution from receptors at the post-synaptic locus, the longer rise-times and steeper slopes of neurons compared with HEK cells suggest a postsynaptic influence on the presynaptic release apparatus. The range of α values apparent in neurons likely reflects heterogeneity in the composition and stoichiometry of SNARE proteins and associated molecules. Synaptobrevin 2 knock-out increases both the mEPSC rise-time and the slope of rise-time versus amplitude plots in the calyx of Held (Guo et al., 2015), where the faster rise-time is in keeping with the need for speed in accurate auditory processing (Trussell, 1999). A comparison of synaptic responses in distal versus proximal dendrites revealed a similar correlation between slope and rise-time (Magee and Cook, 2000), which may indicate that the synaptic scaling they reported has a presynaptic component. Synaptobrevin transmembrane domain mutations alter mEPSC rise-times in neurons (Chiang et al., 2018) and HEK cells (Chiang et al., 2021), and increasing SNARE number enlarges fusion pores (Wu et al., 2017; Bao et al., 2018). Plots of mEPSC rise-time versus amplitude, and the association between the slope of these plots and rise-time offer a new approach to the study of synaptic fusion pores.

Data availability

Data files and code are available in the following open access repository <https://github.com/MeyerJackson/Minis-JGP-2024/tree/main>.

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