Spatial Distribution of Calcium Entry Evoked by Single Action Potentials within the Presynaptic Active Zone

Elliot S. Wachman, Robert E. Poage, Joel R. Stiles, Daniel L. Farkas, and Stephen D. Meriney
The Journal of Neuroscience: March 24, 2004
24(12):2877-2855

Presented by
Nick Morsillo, Deanna Nachreiner, Jordon Torok
Introduction

This paper attempts to uncover spatial distribution of calcium influx at the motor nerve terminal of the adult frog
  – specifically, it argues for relatively few calcium channels in the active zones of the terminal, and for a low probability of a given channel opening

Topics to be covered in this presentation:
  – what role does calcium play in neuronal signaling?
  – the physiology of a frog neuromuscular junction (NMJ)
  – materials and methods used by this study to detect and manipulate the influx of calcium at the NMJ
  – results and conclusions drawn from experimental data
  – statistical predictions to support the conclusions
neurons have a resting membrane potential (voltage) – typically -60 to -70mV

when an action potential occurs, a change in potential propagates across the membrane – the voltage across the membrane spikes to as high as 40mV

at the synapse, this change in voltage (depolarization) causes voltage-gated calcium channels to open
Calcium entry at the synapse triggers neurotransmitter release

neurotransmitters diffuse and bind to receptors on the postsynaptic membrane

bound receptors cause a change in the postsynaptic membrane voltage

if the changes are large enough, the postsynaptic cell fires an action potential of its own
Review of Synaptic Transmission (3)
In this study

- We are observing the frog neuromuscular junction
- preferable because of large, regular active zones—easier to study
The frog NMJ
Observing Calcium entry at the NMJ

- we know the general location and purpose of calcium channels in the motor nerve terminal
- we don’t know the spatial distribution of the channels, or their probabilities of opening from an action potential
- the materials and methods of the study were crafted to obtain this information
Methods (1)

- Using the adult frog cutaneous pectoris nerve-muscle preparation
- Calcium-sensitive dye (Calcium Green-1) loaded into nerve
- Calcium bound Green-1 excited by 488nm laser; fluorescence detected at highest resolution possible
Methods (2)

- Stimulation of the motor nerve
Methods (3)

- Images taken in sets of 20
- First 10 images taken without stimulus
  - these are averaged and result subtracted from other images as “resting fluorescence”
- images are pseudocolored as difference pixel intensity divided by resting fluorescence ($\Delta F/F$)
Methods (4)

- delay between stimulation of motor nerve and postsynaptic response known to be ~1-2msec
- 1.5msec delay from stim. and 1msec illumination window chosen for imaging
- these settings provide the best picture of Ca++ as it enters the active zone
what does each pixel represent?

30-200 possible calcium channels in an active zone

7-50 possible calcium channels per AZ pixel
The Question

What is happening within each pixel? How many calcium channels are there, and what is the probability for one to open?

We can’t currently see calcium entry at a higher resolution; we have to infer at pixel resolution.

Four possibilities considered in this paper:

- a) few calcium channels with low probability of opening
- b) few calcium channels with high probability of opening
- c) many calcium channels with low probability of opening
- d) many calcium channels with high probability of opening

The experiments and detailed analysis suggest that at the frog NMJ there are few channels with low probability of opening (a)
Variability between trials

before we do any experimental manipulations, let's look at some raw data

recordings at the same location over multiple trials

this data argues against possibilities b) and d)
Experimental Manipulations

- Add $\omega$-CgTx (Conotoxin) to block some of the voltage-gated calcium channels
- Channel blockage by conotoxin is irreversible
- Conotoxin added in submaximal concentration so not all channels are blocked
- The data suggests a small number of channels with low probability of opening
Is a graded change in intensity detectable?

- It was argued that there must be few calcium channels since we observed an all-or-none change instead of a graded change in pixel intensity.
- How do we know that a graded change is detectable at all? There could be a problem with the methods.
- Need to verify that a graded change is detectable.
Is a graded change in intensity detectable?

- first, try decreasing the extracellular concentration of calcium.
  - this decreases the calcium flux without changing gating
Is a graded change in intensity detectable?

- next, demonstrate that increasing calcium influx can be detected
- use a potassium channel blocker to broaden the presynaptic action potential
- conclude: the experimental setup can detect graded change in intensity, both positive and negative
- again, the data suggests a small number of channels with low probability of opening
Where does modeling fit in?

Can we use mathematical techniques to further the case for few channels and low probability?
Statistical analysis

- $n =$ the number of calcium channels that lie within a pixel (~6-50)
- $\bar{p} =$ the average probability of any given channel opening during a trial (possible range unknown)

What combination of $n$ and $p$ would reproduce the experimental results?
**Statistical Analysis**

- \( r \) = number of channels that open in a trial

\[
p_r = \binom{n}{r} (\bar{p})^r (1 - \bar{p})^{n-r} ; \quad \binom{n}{r} = \frac{n!}{r!(n-r)!}
\]

- \( \bar{r} \) = expected value for \( r \)
- \( p_0 \) = probability of failure

\[
p_0 = (1 - \bar{p})^n
\]

\[
p_{>0} = 1 - p_0 = 1 - (1 - \bar{p})^n
\]

\[
\bar{r}_{>0} = E(r_{>0}) = \sum_{1}^{n} \left( \frac{r \cdot p_r}{p_{>0}} \right)
\]
Statistical Analysis

- $n = 3$
  - $\bar{r} = 0.3$
  - $CV = 1.73$
  - $r_{>0} = 1.11$

- $n = 6$
  - $\bar{r} = 0.6$
  - $CV = 1.23$
  - $r_{>0} = 1.28$

- $n = 24$
  - $\bar{r} = 2.4$
  - $CV = 0.61$
  - $r_{>0} = 2.61$

- $n = 48$
  - $\bar{r} = 4.8$
  - $CV = 0.43$
  - $r_{>0} = 4.83$
Statistical Analysis

- predicted effect of 2-fold reduction of $n$ on probability of failure and average single pixel intensity

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>$\bar{p} = 0.1$</th>
<th>$\bar{p} = 0.2$</th>
<th>$\bar{p} = 0.8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p_0^{n/2}$</td>
<td>6</td>
<td>1.37</td>
<td>1.95</td>
<td>125</td>
</tr>
<tr>
<td>$n$</td>
<td>48</td>
<td>12.5</td>
<td>212</td>
<td>$6 \times 10^{16}$</td>
</tr>
<tr>
<td>$\bar{r}_{&gt;0}^{n/2}$</td>
<td>6</td>
<td>0.864</td>
<td>0.756</td>
<td>0.504</td>
</tr>
<tr>
<td>$n$</td>
<td>48</td>
<td>0.540</td>
<td>0.502</td>
<td>0.500</td>
</tr>
</tbody>
</table>
Conclusion

The goal of this paper was to study the spatial distribution of calcium influx at the frog neuromuscular junction.

Strong evidence was provided to suggest that there are relatively few calcium channels in the active zone of the motor nerve terminal, and that the probability of any given channel opening from an action potential is small.

This conclusion was supported by statistical predictions about $n$ (number of channels) and $p$ (probability of any one opening) given the experimental results.
Relevance

Are vesicles triggered by few or multiple channels?

What would be the effect of turning a subset of channels on and off?
Acknowledgements

I would like to thank
– Deanna Nachreiner and Jordon Torok
– Joel Stiles and John Pattillo
– NIH and NSF
– everyone at BBSI